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16. ABSTRACT

Trichloroethylene (TCI) is a solvent widely used in the industrial degreasing of metals. It has been detected in the ambient air of a variety of urban and non-urban areas of the United States and also, in natural and municipal waters.

The weight of available evidence obtained from both animal and human data suggest that long-term exposure to environmental levels of TCI poses no serious health concern to the general population. No teratogenic potential has been demonstrated for TCI in studies conducted to date with experimental animals. With respect to the mutagenic potential of TCI, the data on pure TCI do not allow a conclusion to be drawn. If TCI is mutagenic, the available data suggest it would be a very weak, indirect mutagen. Based on available animal data, there is sufficient animal evidence for carcinogenicity. Based on the Agency's Proposed Guidelines for Carcinogen Assessment, the overall evidence for TCI would result in a classification of B2, i.e., a probable human carcinogen.

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U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
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Research Triangle Park, NC 27711

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NOTICE

This document has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment, in consultation with an Agency work group, has prepared this health assessment to serve as a "source document" for EPA use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards; however, at the request of the Agency Work Group on Solvents, the assessment scope was expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses is placed in perspective with observed environmental levels.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in some instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

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The Environmental Mutagen Information Center (EMIC) in Oak Ridge, Tennessee, kindly identified literature bearing on the mutagenicity of TCI. Their initial report and subsequent updates were used to obtain papers from which the mutagenicity assessment was written.

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1. EXECUTIVE SUMMARY

Trichloroethylene (1,1,2-trichloroethylene) (TCI) is a solvent widely used in the industrial degreasing of metals. It has no known natural sources. Current U.S. production is estimated at about 130,000 metric tons per year. Of the TCI used in the United States, 80 to 95 percent evaporates to the atmosphere. In addition to the workplace, TCI is found in a variety of urban and nonurban areas of the United States and other regions of the world. It has been measured in ambient air and water. An average ambient air concentration of about 1 part per billion (ppb) would be expected for some large urban centers. Concentrations as high as 32 ppb have been measured in urban centers in the United States, and 47 ppb has been measured in urban Tokyo. Ambient air concentrations of TCI are greatly influenced by the rate and geographic distribution of emissions and the rate of decomposition and transposition in the atmosphere. The average mixing ratio in the troposphere of the northern hemisphere is 11 to 17 parts per trillion (ppt). Reaction with hydroxyl radicals is the principal mechanism by which TCI is scavenged from the atmosphere.

TCI has been detected in both natural and municipal waters in the United States. Up to 403 ppb has been measured in some surface and subsurface waters. In finished drinking water, concentrations have ranged from 1 to as much as 32 ppb. There is no direct evidence of bioaccumulation of TCI in the food chain. Few studies have been made of the ecological consequences of TCI in the environment.

The pharmacokinetics and metabolism of TCI have been studied in man as well as in animals. Inhalation is the principal route of concern by which TCI enters the body. Ingestion of drinking water contaminated with TCI is another important concern. The extent of TCI absorption after oral ingestion is virtually complete; with air exposure, the amount of TCI absorbed increases in proportion to its concentration in inspired air, the respiratory rate, and duration of exposure. TCI distributes widely into body tissues. TCI is eliminated by two major processes, pulmonary excretion of unchanged TCI and liver metabolism to urinary metabolites. At air concentrations of 500 ppm (2690 mg/m^3) or less, humans are estimated to metabolize between 60 and 90 percent of absorbed TCI. The proportion of the TCI dose metabolized in rodents has been demonstrated to decrease at higher doses; that is, metabolism becomes nonlinear and approaches saturation. However, metabolism is linearly proportional to the inhaled concentration dose in man, at least up to about 300 ppm (1614 mg/m^3). There is no evidence that TCI metabolism in man is saturation-dependent. Studies have not been made of TCI metabolism after oral exposure in man. At the levels found or expected in drinking water, virtually all TCI is expected to be absorbed and metabolized. There is persuasive experimental evidence that the metabolic pathways for TCI are qualitatively similar in mice, rats, and humans. In these species, the principal urinary metabolites of TCI that have been identified are trichloroethanol (TCE) and its glucuronide, and trichloroacetic acid (TCA). Minor metabolites have also been identified for each of these species. In the liver, TCI is first metabolized to chloral hydrate and to a reactive epoxide (TCI oxide) by a microsomal P_{450} system. Reactive intermediate metabolites such as TCI epoxide covalently bind to cellular macromolecules, principally protein, and to a much lesser extent, DNA. Hepatotoxicity of TCI has been shown to correlate with the amount of metabolism. Metabolism of TCI is enhanced by drugs (e.g., barbiturates, oral antidiabetic agents) and by other xenobiotics (e.g., PCBs) which induce the hepatic microsomal P_{450} metabolizing system. Heightened toxicity can result from drug interactions known to occur with ethanol, barbiturates, disulfiram, and Warfarin.

Excluding carcinogenicity as an end point, toxicity testing in experimental animals, coupled with limited human data derived principally from excessive exposure, suggests that long-term exposure of humans to environmental (ambient) levels of TCI is not likely to represent a health concern. Behavioral and psychological effects, particularly as they affect psychomotor performance, have been reported at levels of 200 ppm (1076 mg/m^3) in some, but not all, experimental and epidemiologic studies. There is no information that such impairment, representative of dysfunction of the central nervous system (CNS), would occur during chronic, low-level exposures. At levels found or expected in the ambient environment, such an effect would be unlikely. Similarly, dysfunction of the liver or kidneys would not be likely during or as a result of environmental exposures.

Teratogenicity is another health end point for which available data from experimental animals suggest that the conceptus is not uniquely susceptible to TCI. Exposure of rats, mice, and rabbits, during gestation, to levels (300 ppm or 1614 mg/m^3) greatly in excess of those generally found in the environment, has not been observed to result in any teratogenic effects. The teratogenic potential of TCI for humans cannot be directly extrapolated from the observations in the animal studies. However, the animal studies suggest that at low ambient levels that do not cause maternal toxicity, TCI would not pose a significant hazard to the developing conceptus.

With respect to the mutagenic potential of TCI, available data provide suggestive evidence that commercial-grade TCI is a weakly-active, indirect mutagen, causing effects in a number of different test systems representing a wide evolutionary range of organisms. Thus, commercial TCI may have the potential to cause weak or borderline increases above the spontaneous level of mutagenic effects in exposed human tissue. Adverse effects in the testes of mice exposed to commercial TCI suggest that TCI could cause similar effects in humans. A conclusion about the mutagenic potential of pure TCI cannot be made. If TCI is mutagenic, the available data suggest that it would be a very weak, indirect mutagen.

The evidence reviewed in this document for the carcinogenicity of TCI in experimental animals includes: increases in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice (three studies); malignant lymphomas in female Han:NMRI mice; and renal adenocarcinomas in male Fischer 344 rats. Statistically significant increases of malignant liver tumors were observed in both male and female B6C3F1 mice. A gavage study of purified TCI in both sexes of B6C3F1 mice was conducted with basically the same experimental design as a gavage study of technical grade TCI in male and female B6C3F1 mice to evaluate the role of epoxide stabilizers in TCI in the induction of hepatocellular carcinomas. Similar carcinogenic responses were observed for the purified epoxide-free and for stabilized TCI. The other studies provide some additional support to the overall body of evidence, particularly since two of them were carried out in a different species or strain. However, the third study in B6C3F1 mice, an inhalation study in which an increase in liver tumors was seen, was weakened by deficiencies in its conduct. In the inhalation study in Han:NMRI mice, a 30 percent incidence of spontaneous lymphoma in control mice, and the possibility of an indirect effect in treated mice, made these results difficult to interpret. A borderline response was observed in the gavage study of Fischer 344 rats.

A gavage study with male and female Osborne-Mendel rats was negative; however, this study may be considered inconclusive because of high mortality in the treatment groups. Exposure to airborne concentrations of TCI did not result in a carcinogenic effect in Han:Wist rats and Syrian hamsters, although higher dose levels of TCI probably could have been given to the test animals.

Other long-term animal studies did not suggest a carcinogenic potential for TCI. Most of these studies, however, were not specifically designed to evaluate carcinogenicity, involved only small treatment groups, lacked sufficient doses of TCI, or were of insufficient duration in view of the expected latency period for cancer induction.

TCI induced malignant tumors of the liver in both male and female B6C3F1 mice in multiple studies. This constitutes a signal that TCI might be carcinogenic in man. While kidney tumors observed in the NTP rat study are not strong indications of a response in a second species because of the small number of animals responding (3 of 49 animals) and because of the high mortality, the statistical significance after mortality corrections suggests that a carcinogenic effect may be taking place.

The U.S. Environmental Protection Agency's Proposed Guidelines for Carcinogen Assessment (U.S. EPA, 1984), take the position that the mouse-liver-tumor-only response, when other conditions for a classification of "sufficient" evidence in animal studies are met, should be considered as "sufficient" evidence of carcinogenicity. Thus, based on EPA's proposed cancer guidelines, the overall evidence for TCI would result in a classification of B2, i.e., a probable human carcinogen.

The TCI carcinogenicity results could be classified under the criteria of the International Agency for Research on Cancer (IARC) as either "sufficient" or "limited" depending on which of the differing current scientific views about chlorinated organic compound induction of liver tumors in mice is chosen. Since there are no adequate epidemiologic data in humans, the overall ranking of TCI under the criteria of the IARC depends primarily upon the position taken regarding the mouse liver tumor. Thus, the overall ranking could be either Group 2B or Group 3. The more conservative public health view would regard TCI as a probable human carcinogen (Group 2B), but there is also scientific sentiment for regarding TCI as an agent that cannot be classified as to its carcinogenicity for humans (Group 3).

Additional carcinogenicity studies on purified TCI (no detectable epoxides) are currently in progress. These studies and others published recently will be incorporated into any updates of this Health Assessment Document.

Four sets of gavage bioassay data on hepatocellular carcinomas in male and female mice provide a basis to calculate an upper-bound carcinogenic slope estimate for TCI using a linearized multistage model. The development of these risk estimates is for the purpose of evaluating the "what-if" question: If TCI is carcinogenic in humans, what is the possible magnitude of the public health impact? Since upper-bound potency estimates calculated on the basis of these data sets are comparable, ranging from 5.8×10^{-3} to 1.9×10^{-2} mg/kg/day, the geometric mean, 1.1×10^{-2} mg/kg/day is used to calculate the incremental lifetime cancer risk (i.e., unit risk) due to a unit exposure of TCI in drinking water and in air. The upper-bound estimate of the cancer risk due to 1 μg/L of TCI in drinking water is 3.2×10^{-7}. The upper-bound estimate of the cancer risk due to 1 μg/m^3 of TCI in air is 1.3×10^{-6}. The upper-bound nature of these estimates is such that the true risk is not likely to exceed this value and may be lower. The carcinogenic potential of TCI is generally considered to reside in cellular reactive intermediate metabolites, and therefore metabolic and pharmacokinetic factors have been used in the calculation of the drinking water and air unit risks.

None of the epidemiologic studies reviewed in this report provide positive evidence from which to estimate a unit risk for exposure to TCI. As an alternative, an upper-bound estimate has been calculated from one negative study. The calculation on the basis of human data results in a greater risk estimate than the estimate from animal data, and thus does not appear to contradict the risk estimate calculated from the animal data.

Expressed in terms of relative potency, TCI ranks in the lowest quartile among the 54 suspect or known human carcinogens evaluated by EPA's Carcinogen Assessment Group.

RECOMMENDATIONS FOR FURTHER STUDIES

Areas for which incomplete information is available, and which should be considered in formulating research needs, are presented below. The needs listed, however, are not necessarily in the order of relative priority.

1. Teratogenicity and Reproductive Effects. There are few animal studies via inhalation that adequately assess the teratogenic and reproductive effects potential of TCI. Uncertainty about these biological end points in humans should serve as a stimulus for future research.

2. Neurobehavioral Toxicity. There have been few animal studies of the effects of TCI on the nervous system and behavior. Most end points studied have been relatively insensitive. Further studies are warranted on more sensitive end points.

2. INTRODUCTION

Trichloroethylene (TCI) is a member of a family of unsaturated chlorinated aliphatic compounds. Trichloroethylene, though a water and solid waste contaminant, is primarily of interest in situations involving ambient air exposure. It is released into ambient air as a result of evaporative losses during production, storage, and/or use. It has no known natural sources. It is photochemically reactive in the troposphere and is removed by scavenging mechanisms, principally via hydroxyl radicals.

The scientific data base concerning the effects of TCI on humans is limited. Effects on humans have generally been ascertained from studies involving individuals occupationally or accidentally exposed. During such exposures, the concentrations associated with adverse effects on human health were either unknown or far in excess of concentrations found or expected in ambient air. Controlled TCI exposure studies have been directed toward elucidating the effects on the CNS, effects on clinical chemistries, and pharmacokinetic parameters of TCI exposure.

The available epidemiologic studies have not been able to adequately assess the overall impact of TCI on human health. It has therefore been necessary to rely greatly on animal studies to derive indications of potential harmful effects. Although animal data cannot always be extrapolated to humans, indications of probable or likely effects in animal species increase confidence that similar effects may occur in humans.

This document is intended to provide an evaluation of the scientific data base concerning TCI. The publications cited in this document represent a majority of the known scientific references to TCI. Reports which had little or no bearing on the issues discussed were not cited. Some topic areas, such as effects on terrestrial ecosystems, are only briefly discussed. Such topics reflect the nature of the scientific data base. On-going literature searches have been conducted through 1985, resulting in the inclusion of selected references in this document. However, the basic literature search for Chapter 8 is current through 1982; the basic literature search for Chapter 7 is current through 1983. The Agency is aware of additional carcinogenicity and mutagenicity information that has subsequently become available. This information will be considered for incorporation in any future updates of this document.

3. BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Trichloroethylene (TCI) is a colorless, highly volatile liquid that is miscible with a variety of solvents. Some of its important physical properties are shown in Table 3-1.

TABLE 3-1. PHYSICAL PROPERTIES OF TRICHLOROETHYLENE

Molecular weight	131.39
Boiling point	87°C
Vapor pressure	94 mm Hg @ 30°C
Vapor specific gravity	4.55 at boiling point (air = 1)
Solubility @ 20°C in water	0.107 g percent

At a temperature of 25°C and a pressure of 1 atmosphere, one part-per-million (ppm) of the vapor is equivalent to 5.38 mg/m^3 in air.

Trichloroethylene is photochemically reactive and autooxidizes upon catalysis by free radicals (McNeill, 1979). Autooxidation is greatly accelerated by high temperatures and exposure to ultraviolet radiation (McNeill, 1979). Some of its degradation products, e.g., hydrochloric acid (HCl), are corrosive to metals. These decomposition products include dichloroacetyl chloride, phosgene, carbon monoxide, hexachlorobutene, and HCl (McNeill, 1979.)

Decomposition is catalyzed when TCI comes in contact with aluminum metal. The HCl produced reacts with aluminum to yield aluminum chloride ($AlCl_3$) which catalyzes formation of hexachlorobutene (McNeill, 1979). Sufficient quantities of aluminum have been reported to cause violent decomposition of TCI (Metz and Roedy, 1949).

While TCI is nonflammable under ordinary conditions, mixtures of TCI and oxygen will ignite at 25°C when the TCI concentration is between 8 to 10 percent in air (McNeill, 1979).

To inhibit its decomposition, stabilizers are added to commercial formulations of TCI (McNeill, 1979). Commonly used stabilizers in vapor degreasing grades of TCI are neutral inhibitors and free radical scavengers (McNeill, 1979).

Epoxides (including epichlorohydrin, a known animal carcinogen) are added to scavenge free HCl or $AlCl_3$. Antioxidants are usually added in quantities less than 1 percent by weight (Hardie, 1964). Fuller (1976) reported that inhibited grades of TCI are stable up to 130°C in the presence of air, moisture, light, and common construction metals. At higher temperatures, inhibitors are less effective and corrosion of metals can result. Stabilizers that have been used in TCI formulations are shown in Table 3-2.

TABLE 3-2. STABILIZERS IN TRICHLOROETHYLENE FORMULATIONS (Beckers, 1972; U.S. EPA, 1975; Hardie, cited in: Kirk Othmer, 1975; Schlossburg, 1980)

Amyl alcohol
Propanol
Diethylamine
Triethylamine
Dipropylamine
Diisopropylamine
Diethanolamine
Morpholine
N-Methylmorpholine
Aniline
Acetone
Ethyl acetate
Borate esters
Ethylene oxide
1,2 Propylene oxide
1,2 Epoxy butene
Cyclohexene oxide
Propylene oxide
Butadiene dioxide
Styrene oxide
Pentene oxide
2,3 Epoxy 1-propenol
3, Methoxy-1,2 epoxy propane
3, Ethoxy-1,2 epoxy
Stearates

2-Methyl-1,2 epoxy propanol
Epoxy cyclopentanol
Epichlorohydrin
Tetrahydrofuran
Tetrahydropyran
1,4-Dioxane
Dioxalane
Trioxane
Alkoxyaldehyde hydrazones
Methylethyl ketone
Nitromethanes
Nitropropanes
Phenol
o-Cresol
Thymol
p-tert-Butylphenol
p-tert-Amylphenol
Isoeuganol
Pyrrole
n-Methyl pyrrole
n-Ethyl pyrrole
(2-pyrryl) Trimethylsilane
Glycidyl acetate
Isocyanates
Thiazoles

Trichloroethylene decomposes at temperatures up to 700°C. Substances found in the decomposition mixture include dichloroethylene, tetrachloroethylene, carbon tetrachloride, chloroform, and methyl chloride (Hardie, 1964). Formation of phosgene, a highly toxic gas, was observed when TCI came in contact with iron, copper, zinc, or aluminum over the temperature range 250°C to 600°C (Sjöberg, 1952). When TCI came in contact with zinc at 450°C, 69 mg phosgene per gram of TCI was produced.

Trichloroethylene is not readily hydrolyzed by water (McNeill, 1979). In the presence of strong alkali (e.g., sodium hydroxide) TCI can decompose to dichloroacetylene, an explosive, flammable, and highly toxic compound (Humphrey and McClelland, 1944). Noweir et al. (1973) recommended that TCI be stored in cans or dark glass bottles to minimize decomposition.

Because of its lipophilic properties, TCI can be expected to partition selectively in the body's adipose tissue. The relationships between such partitioning and manifestation of its toxic properties are discussed in Chapter 4. The unsymmetrical nature of the molecule and the electron-withdrawing effect of the chlorine substituents (leading to destabilization of the charge at the double bond) have been proposed as factors responsible for the supposed mutagenicity of TCI (Bonse and Henschler, 1976).

Measurements of the lipophilic potential of TCI have been made by a number of investigators (Waters et al., 1977; Mapleson, 1963; Powell, 1947; Sato and Nakajima, 1979). Mapleson (1963) reported an oil/water partition coefficient of 960:1 at 37°C. Waters et al. (1977) reported the oil/water coefficient as 900:1. Sato and Nakajima (1979) reported an olive oil/water partition coefficient of 552:1 at 37°C and an olive oil/blood partition coefficient of 76:1. The relationship of TCI to other compounds with respect to partition coefficients is shown in Table 3-3 (Sato and Nakajima, 1979). These values indicate that TCI is highly lipophilic.

3.2 ENVIRONMENTAL FATE AND TRANSPORT

3.2.1 Production

Trichloroethylene has been declining in production in recent years because of restrictions on emissions (Federal Register, 1977) and substitution by other solvents. According to statistics published by the U.S. International Trade Commission (1982), 117,363 metric tons of TCI were produced in 1981.

Recent estimates of world production of TCI are not available. Such figures for 1973 indicated 700,000 metric tons were produced (McConnell et al., 1975). About two-thirds was produced outside the United States.

In 1977, TCI was produced by four American manufacturers (SRI, 1982). The producers and production capacities are shown in Table 3-4.

Most of the TCI produced commercially is derived from ethylene or dichloroethane (EDC) (McNeill, 1979). Dichloroethane, produced by chlorination of ethylene, can be further chlorinated to TCI and

TABLE 3-3. PARTITION COEFFICIENTS OF TRICHLOROETHYLENE AND OTHER CHLORINATED HYDROCARBONS (Sato and Nakajima, 1979)

Solvent	Oil/water	Oil/blood	Log P (partition coefficient
Dichloromethane	21	16	1.32
Chloroform	115	39	2.06
Carbon tetrachloride	1444	150	3.16
1,2-Dichloroethane (EDC)	40	23	1.60
Methyl chloroform	383	108	2.58
Tetrachloroethylene	4458	146	3.65
Trichloroethylene	552	76	2.74

TABLE 3-4. U.S. PRODUCTION CAPACITY FOR TRICHLOROETHYLENE (SRI, 1982)

Producer	Site	Annual capacity, Jan. 1981 (metric tons)
PPG Industries	Lake Charles, LA	91,000
Dow Chemical	Freeport, TX	54,000
Diamond Shamrock	Deer Park, TX	23,000
Ethyl Corporation	Baton Rouge, LA	20,000

tetrachloroethylene at 280° to 450°C. Catalysts include potassium chloride, aluminum chloride, fuller's earth, graphite, activated carbon, and activated charcoal (McNeill, 1979). Maximum conversion to TCI (75% of EDC feed) is achieved at a chlorine-to-EDC ratio of 1.7:1 (McNeill, 1979).

Oxychlorination of ethylene or EDC to TCI is achieved at 425°C and at 20 to 30 psi (McNeill, 1979). Common catalysts are mixtures of potassium and cupric chlorides. Conversion efficiency is between 85 and 90 percent.

3.2.2 Use

It has been estimated that from 80 to 95 percent of the TCI produced in the United States is used in the degreasing of fabricated metal parts (SRI, 1978; McNeill, 1979; Chemical and Engineering News, 1975). The remaining 5 to 20 percent is divided equally between exports and miscellaneous applications. Use in vapor degreasing is estimated to account for 87 percent of production, according to industry trade statistics (Chemical Marketing Reporter, 1978).

In addition to its use as a vapor degreasing agent, TCI has a wide variety of other uses (Table 3-5). About 4,500 to 6,800 metric tons are consumed annually in the production of polyvinyl chloride, in which TCI is used as a chain-transfer agent (McNeill, 1979).

TABLE 3-5. MISCELLANEOUS USES OF TRICHLOROETHYLENE
(McNeill, 1979; Waters et al., 1977)

Adhesive formulations
Paint-stripping formulations
Low-temperature heat-transfer medium
Carrier solvent in industrial paint systems
Solvent in textile dyeing and finishing

Uses of TCI that have been discontinued in the United States because of environmental and health restrictions include use as an inhalation anesthetic (Page and Arthur, 1978; Waters et al., 1977), use in fumigant mixtures (Farm Chemicals Handbook, 1976), and use as an extractant in the decaffeination of coffee. Use in anesthetic procedures may be continuing to some extent in other countries (Cundy, 1976).

Declining consumption of TCI from the early 1970's has been offset by the increased use of substitute solvent, e.g., methyl chloroform and methylene chloride (U.S. EPA, 1979).

3.2.3 Emissions

There are no known natural sources of TCI. Graedel and Allara (1976) concluded that atmospheric chemical production of TCI was negligible. Because of its dispersive use pattern in degreasing operations, most of the TCI used is expected to be emitted to the atmosphere. Emissions estimates (U.S. EPA, 1979) for 1976, pertaining to vapor degreasing, cold cleaning, and miscellaneous use categories suggest that 130,000 metric tons were discharged to the atmosphere.

Solvent emissions from vapor degreasing occur primarily during unloading and loading of the degreaser (Bucon et al., 1978). Daily emissions of a single spray degreasing booth may vary from a few pounds to 1,300 pounds (Bucon et al., 1978). In uncontrolled degreasing operations, as much as 2.8 kg/hr can be emitted, based on 200,000 pounds of metal cleaned per day (Bucon et al., 1978).

Singh et al. (1979) estimated global emissions of TCI for 1977 at 435,000 metric tons ± 50 percent. Estimates were derived from production figures and usage patterns.

3.2.4 Persistence

Many processes occur in the troposphere which can alter the atmospheric levels of TCI. Once emitted into the troposphere, vertical and horizontal mixing occurs. Transport is highly dependent upon the length of time TCI remains in the troposphere, which is determined largely by the extent to which TCI reacts with hydroxyl free radicals (·OH), the principal scavenging mechanism for TCI and many other halogenated compounds in the atmosphere.

Edney et al. (1983), on the basis of the observed rate of reaction of TCI with ·OH in a reaction cell, calculated an atmospheric lifetime for TCI of about 54 hours. An ·OH concentration in the troposphere of 10^6 molecules/cm^3 was assumed.

The average northern hemisphere background mixing ratio is between 11 and 17 ppt (Singh et al., 1979), which suggests that TCI is short-lived in the troposphere. Singh et al. (1979) estimated a residence time of about 2 weeks, an estimate based on a seasonally-averaged ·OH concentration of 4×10^5 molecules/cm^3 and a rate constant (National Bureau of Standards, 1978), at 265°K, of 2.30×10^{-12}. Singh and coworkers (1979) estimated that, given an ·OH concentration of 1×10^6 molecules/cm^3, 20 percent of the TCI in ambient air can be destroyed each day. Crutzen et al. (1978) estimated a residence time of 11 days for TCI. Derwent and Eggleton (1978) estimated the lifetime at 0.04 year (~ 15 days). The percentage of the ground level injection of TCI that was estimated to survive free radical attack was 0.4 percent.

Seasonal variations in ·OH concentrations, important for longer-lived species, are not expected to play a significant role in the tropospheric survival of TCI. Altshuller (1980) calculated that in January (when ·OH levels and solar flux are low) 0.6 day would be required for the photochemical decomposition of 1 percent of the ambient TCI. In July (when ·OH levels and solar flux are high), only 0.07 day is required.

Trichloroethylene was observed to have a slow decomposition rate in dilute aqueous solution (Dilling et al., 1975). Its half-life (in the dark) was 10.7 months at ambient temperatures. When the solution was exposed to sunlight, 75 percent of the TCI decomposed in 12 months, compared with 54 percent for the reaction in the dark. A correction for the amount of TCI that volatilized into the air space above the solution was not employed. On the other hand, Pearson and McConnell (1975), in their determinations of the hydrolytic decomposition, extrapolated to zero volatilization. Their estimate was a half-life of 30 months.

The major route of removal of TCI from water is volatilization. Volatilization of TCI from water has been investigated by several authors. Dilling et al. (1975) have shown that the loss of TCI from an agitated dilute aqueous solution occurs exponentially with an evaporative half-life of 21 ± 3 minutes. Scherb (1978) measured the volatilization of TCI from an aeration channel in a wastewater treatment plant and found the half-life to be 3 hours. The rate of volatilization of TCI from surface waters in the environment has been reported by Zoeteman et al. (1980). TCI was found to have a half-life of 1 to 4 days in the Rhine River and 30 to 40 days in a tidal estuary. Smith et al. (1980) have shown that the rate of volatilization of TCI and other low-molecular-weight compounds from various bodies of waters is a function of reaeration rates. From the work of Smith et al. (1980), it is estimated that the half-life for TCI in surface waters ranges from 3 hours, for rapidly moving shallow streams, to 10 days or longer for ponds and lakes (Table 3-6).

TABLE 3-6. ESTIMATED HALF-LIFE OF TCI IN SURFACE WATERS

Water Type	TCI half-life (days)
Pond	11
Lake	4 to 12
River	1 to 12

Source: Calculated from information in Smith et al. (1980).

3.2.5 Degradation

The transformation products of the decay of TCI have been investigated in chamber studies simulating atmospheric conditions. Two principal transformation products have been observed, phosgene and dichloroacetyl chloride. Dahlberg (1969) observed that both were produced when TCI was photooxidized in air. Gay et al. (1976) also found formyl chloride, in addition to phosgene and dichloroacetyl chloride, when TCI was photooxidized in the presence of nitrogen dioxide (NO_2). A maximum observed consumption rate for TCI of 70 percent per hour was reported. In this system, dichloroacetyl chloride, identified by long-path infrared spectroscopy, was the principal product. In a smog chamber system containing 2.5 ppm TCI and 0.5 ppm NO_2, TCI disappeared at the rate of about 25 percent per hour (Dimitriades et al., 1983). Ozone built up to 0.5 ppm. The presence of equal amounts of tetrachloroethylene and TCI in a chamber containing NO_2 did not alter the consumption rate of TCI above TCI controls.

Edney et al. (1983) observed a number of degradation products when ·OH radicals reacted with TCI in a 700 liter cell coupled to an FTIR spectrometer and scanning interferometer. Reaction products observed were phosgene, dichloroacetyl chloride, formyl chloride, carbon monoxide, and nitric acid. The rate constant of reaction was 3.6×10^{-12} cm^3/sec.

Lillian et al. (1976), finding phosgene formation from the photooxidation of the TCI congener, tetrachloroethylene, considered that phosgene formation also was likely from other chloroethylenes. Singh (1978) reported that ·OH-catalyzed photodecomposition of TCI, as occurs in the troposphere, can result in phosgene as the principal transformation product.

Under simulated atmospheric conditions in the presence of nitric oxide (NO) and NO_2, TCI (10 ppm) was very short-lived (less than 3.5 hours) (Dilling et al., 1976). A consumption rate up to 30 percent per hour was reported. The high reactivity of TCI and its catalytic role in the formation of photochemical smog have been discussed (U.S. EPA, 1978a; National Academy of Sciences, 1977). Free radicals (e.g., $\cdot RO_2$), are formed when TCI is photodecomposed, and these in turn react with NO by oxidizing it to NO_2. According to the following reaction sequence, NO_2 results in the formation of ozone (O_3):

$$(1) \quad NO_2 \xrightarrow{hv} NO + O$$

$$(2) \quad O + O_2 + M \text{ (third body)} \longrightarrow O_3 + M$$

Because chamber studies simulate some atmospheric reactions believed to occur in the environment, further studies of greater complexity would be needed to determine the extent and pathways by which TCI is photodecomposed. Phosgene and dichloroacetyl chloride, though likely to be formed in the troposphere, also are subject to transformation and scavenging processes.

3.3 LEVELS OF EXPOSURE

3.3.1 Analytical Methodology

Of the analytical methods and procedures available for detecting and quantitating halogenated hydrocarbons present in ambient air, water, soil, foods, etc., two systems are commonly used: gas chromatography/mass spectrometry

(GC-MS) and gas chromatography with electron capture detection (GC-ECD). Each system has the capability of detecting TCI concentrations as low as a few parts per trillion by volume. The usefulness of GC-ECD is that the apparatus can be used in the field to provide quasi-continuous measurements by intermittent sampling (every 15 to 20 minutes). Such use can circumvent decomposition that results when samples of TCI are stored for long periods before assay (Pellizzari, 1974; Lovelock, 1974b).

3.3.1.1 Gas Chromatography with Electron Capture Detection. The GC-EC methodology has been reviewed by Pellizzari (1974) and by Lovelock (1974b). The EC detector is specific in that halogenated compounds are quantified while non-halogenated hydrocarbons are not detected. Thus, high background levels of non-halogenated hydrocarbons in ambient air or water samples do not interfere with measurements of halogenated compounds. In a complex mixture in which several compounds may have similar retention times, alteration of the operating parameters of the GC-EC system will usually provide separation of the components.

In the method developed by Rasmussen et al. (1977), the detection limit for TCI was 0.3 ppt. With four samples of ambient air, the percent standard deviation was 23.0 by electronic area integration (+ 5 percent by peak height measurement). The system, which employs a freezeout concentration loop, has the capability of measuring various halocarbons in 500-ml aliquots of air. Total analysis time is 40 minutes. The GC column consisted of a 6-mm x 3-m stainless steel column packed with 10 percent SF-96 on 100/120 mesh-Chromosorb W.

A similar analytical procedure was employed by Harsch and Cronn (1978) to measure levels of halocarbons in 30-ml samples of low-pressure stratospheric air. For TCI, the detection limit was 1 ppt and the precision (percent standard deviation) for 10 samples with a mixing ratio of 22 ppt was 10 percent.

Gas chromatography with electron capture detection also has been the method of choice by Singh and coworkers for measuring the ambient air levels of a wide variety of halocarbons. Singh et al. (1979) maintained the EC detector at 325°C. The GC column used was 6-ft x 1/8-in. stainless steel, containing 20 percent SP2100, 0.1 percent Carbowax 1500 on 100/120 mesh Supelcoport. Singh et al. (1979b) recommended two columns for separation of TCI: (a) silicone oil 10 percent DC 200 on 30/60 mesh Chromosorb W in a 6-ft x 1/4-in. stainless steel column or (b) 20 percent SP-2100 on 80/100 mesh Supelcoport in a 15-foot x 1/8-inch column.

The applicability of GC-ECD to analysis of TCI and other halohydrocarbons purged from water samples has been discussed by Bellar et al. (1979). Preconcentration on a Tenax and silica gel trap was recommended.

Singh et al. (1979a) avoided the use of Tenax traps for preconcentrating organics in air samples because of artifacts. They thought that oxygen or ozone was oxidizing the Tenax monomer and producing electron-absorbing oxygenated species, thus interfering with EC detection of the organics of interest.

Makide et al. (1980) used GC, coupled with constant current ECD, to measure TCI in ambient air at sites in Japan. An extremely low detection limit of <0.2 ppt ($<1.36 \times 10^{-6}$ mg/m^3) was reported. Sampling canisters were made of stainless steel and were polished electrochemically. Preconcentration was carried out on a silicone OV-101 column at -40°C. Column temperature was raised at a rate of 5°C per minute up to 70°C for separation of components.

A direct headspace technique for use with EC detection has been reported by Piet and coworkers (1978). This technique avoids the need for preconcentration traps when water samples are purged. An overall detection limit below 1 µg/l was reported. With isothermal operation of the GC, a detection limit for TCI of 0.05 µg/l was reported. An OV225 capillary column was used.

Dietz and Singley (1979) described a headspace method applicable to EC detection of volatile organics from water samples. A standard deviation of 5 percent was reported for routine analyses of drinking, natural, and industrial waters. Concentrations ranging from 0.1 ppb to several ppm of TCI were detected. In this method, the water sample is sealed in a vial until organics equilibrate between the water and vial headspace. A major advantage is that only relatively volatile, water-insoluble compounds tend to partition in the headspace. In a comparison of this method with the purge and trap technique, Dietz and Singley (1979) obtained comparable results. However, the chromatograms from the purge-trap method were of poorer quality. The headspace method was recommended for routine sample analyses of volatile halocarbons.

Wang and Lenahan (1984) described a closed-loop system that combines the techniques of both gas stripping and headspace, with a reported detection limit of about 0.2 µg/l.

3.3.1.2 Gas Chromatography-Mass Spectrometry. While GC-ECD relies on retention time and ionization efficiency for compound identification, the equally sensitive GC-MS system identifies compounds by their characteristic mass spectra. However, the expense, bulk, and demands on the operator preclude routine use of GC-MS in field measurements. Often, GC-MS is used to verify results obtained by GC-ECD.

Comparisons between the precision afforded by GC-MS versus GC-ECD have been made by Cronn and co-workers (1976). In these comparisons, the detection limit was defined based on a peak height three times the noise level with a 75-ml sample loop. With TCI at an average mixing ratio of 128 ppt, the detection limit (GC-MS) was 23 ppt (with 3 doped samples of zero air). The percent standard deviation was 10. More recent data from Cronn and Harsch (1979) show a slightly lower detection limit (16 ppt) for a 100-ml freezeout sample. The sensitivity of the GC-MS technique was achieved using direct coupling of the gas chromatograph and mass spectrometer and selective ion monitoring (at mass 95 for TCI).

Pellizzari et al. (1976) used Tenax GC to absorb TCI and other halogenated hydrocarbons from ambient air. Recovery was made by thermal desorption and helium purging into a freezeout trap. The estimated detection limit when high resolution GC-MS is used is 1.92 ppt (0.01×10^3 mg/m^3). Accuracy was reported at ±30 percent. Included among the inherent analytical errors were (1) the ability to accurately determine the breakthrough volume, (2) the percent recovery from the sampling cartridge after a period of storage, and (3) the reproducibility of thermal desorption from the cartridge and its introduction into the analytical system. To minimize loss of sample, cartridge samplers should be enclosed in cartridge holders and placed in a second container that can be sealed, protected from light, and stored at 0°C. The advantages reported for Tenax include (1) low water retention, (2) high thermal stability, and (3) low background levels (Pellizzari, 1974; Pellizzari, 1975; Pellizzari, 1977; Pellizzari et al., 1976).

In an analytical system using Tenax GC as the absorbent, Krost et al. (1982) reported an estimated detection limit for TCI of 2 ppt. Analysis of the breakthrough volumes at various temperatures suggests that Tenax GC is not a particularly effective absorbent for TCI.

Resolution of TCI from other hydrocarbons was reported to be effective when Carbopak C-HT, a graphitized thermal carbon black treated with hydrogen at 1,000°C was used as the adsorbent in a GC column (Knoll et al., 1979). Flame ionization was used in detection. Interference between TCI and ethylene dichloride was reported with Porapak T.

Gas chromatography-mass spectrometry has been more widely used in measurements of volatile organic compounds in aqueous samples. In this regard, gas purging followed by trapping on an absorbent has been extensively used in combination with GC-MS detection. A review of this system, as applied to TCI and other volatile organics, has been made by Bellar et al. (1979). A combination of Tenax GC (60/80 mesh) and silica gel was recommended. Purging was accomplished at a water temperature of about 22°C. In general, the method is applicable to compounds that have a low solubility in water and a vapor pressure greater than water at ambient temperatures. For well-defined samples when the probability of unexpected compounds is low, the EC detector could be used. The detection limit of this system was reported to be in the 0.1 to 1 μg/L range. Desorption onto the head of the column can be accomplished in one step by heating.

3.3.2 Calibration

Both static and dynamic procedures have been used to calibrate the instrumentation used for measurement of TCI. Because of the very low levels of TCI found or expected in ambient air, the generation of known low-level concentrations of TCI is critical in instrument calibration.

Singh and coworkers (1979, 1977) employed permeation tubes to calibrate their GC-EC instrumentation. A tube constructed of FEP Teflon (wall thickness of 0.03 in.) was found to be satisfactory for TCI. Errors in the permeation rate were less than 5 percent (Singh et al., 1979). It was considered the best, most accurate means of generating primary standards. Since primary standards could not be used in field operations, secondary standards were generated and compared to them. The permeation rate at the 95-percent confidence level was 246.0 ± 20.1 mg/min (Singh et al., 1977).

Primary and secondary standards prepared by multiple dilutions were used by Cronn and coworkers (1976, 1977a,b).

3.3.3 Sampling and Sources of Error

Common approaches used to sample ambient air for trace gas analysis are presented below (National Academy of Sciences, 1978).

(1) Pump-pressure samples: A mechanical pump is used to fill a stainless steel or glass container to a positive pressure relative to the surrounding atmosphere.

(2) Ambient pressure samples: An evacuated chamber is opened and allowed to fill until it has reached ambient pressure at the sampling location. If filling is conducted at high altitude, the container may easily become contaminated when returned to ground level.

(3) Adsorption on molecular sieves, activated charcoal, or other adsorbents.

(4) Cryogenic samples: Air is pumped into a container and liquefied, and a partial vacuum is created which allows more air to enter. This method allows for the collection of several thousand liters of air.

Singh and coworkers (1979) employed electropolished stainless steel vessels in their sampling of tropospheric air. Vessels were checked for background contamination until it was reduced to less than 2 to 3 percent of the expected background concentration of a given trace substance. Typically, vessels that maintained positive pressure were not affected by room contamination.

Sampling and storage of ambient air collected at stratospheric altitudes present more of a problem. Because of the low pressures in the sampling vessels, contamination may easily result when vessels are returned to ground level. Cronn and Robinson (1978) and Harsch and Cronn (1978) reported that contamination of low-pressure stainless steel vessels precluded use of data for TCI. Whole-air samples were collected at stratospheric altitudes. Contamination occurred during the sample-transfer procedure because of high mixing ratios of halocarbons in laboratory air.

To overcome contamination of low-pressure samples, Harsch and Cronn (1978) developed a low-pressure sample-transfer technique. A vacuum transfer procedure employing a freezeout loop (Rasmussen et al., 1977) was found to yield results with sufficient sensitivity and with minimal contamination problems. A leak-tight system was considered essential. System components were silver-soldered.

Sample collection and handling and preservation of water samples have been discussed by Kopfler et al. (1976) and by Bellar et al. (1974). Narrow-mouth vials with a total volume in excess of 50 ml were considered acceptable (Bellar et al., 1974). It was recommended that the vials be filled to overflowing and each covered with a Teflon-faced silicone rubber septum. Because of the presence of chlorine in some drinking water supplies and the chlorination of organic materials, an increase in the concentration of some halocarbons may occur also during storage (Kopfler et al., 1976).

Bellar et al. (1974) reported that free chlorine can result in the formation of TCI and other halohydrocarbons. In measurements of TCI in water from a sewage-treatment plant, they found an increase of 1.2 µg TCI/L in the water after chlorination. Dietz and Singley (1979) collected water samples in 125-mL serum vials that had been cleaned by detergent wash, distilled water rinse, dichromic acid wash, distilled water rinse, acetone rinse, hexane rinse, acetone rinse, distilled water rinse, and oven-drying at 150°C. Vials were completely filled and then sealed with Teflon-faced septa and aluminum crimp seals. Samples were stored at 4°C. Minimum loss occurred during storage at 4°C up to 28 days.

The stability of stored samples of TCI present in ambient air or breath samples from exposed individuals has been investigated by Pasquini (1978). The mean percent loss of TCI in alveolar breath samples collected in glass tubes at room temperature was 59.3 ± 15.3 percent over 169 hours. The mean decay rate of TCI in dilution air samples was 4.7 ± 3.2 percent over the same time interval. Moisture, temperature, and tube surface and condition can alter greatly vapor retention. For TCI, tubes stored at 37°C had high vapor retention rates. When breath was exhaled into the tubes at 37°C, water vapor was not condensed from the sample. Additional experiments with TCI indicated that siliconized tubes showed a lower decay rate than non-siliconized tubes.

Renberg (1978) judged XAD-4 the most suitable of several possible adsorbents for volatile halogenated hydrocarbons in water, including TCI. This method results in an extract concentrated enough for both chemical determination and small-scale biological tests.

Kummert et al. (1978) have suggested a direct aqueous injection high-pressure liquid chromatography technique for analysis of TCI in natural waters. The technique has been successfully applied to an actual water pollution case for tetrachloroethylene. The detection limit for TCI was reported as 1 µmol/ℓ for a 10-ml sample.

3.3.3.1 Air-Mixing Ratios. Measurements of ambient air mixing ratios of TCI have been made at a variety of sites in the United States and abroad. As shown in Table 3-7, urban areas have mixing ratios in the low ppb range.

Singh et al. (1979) reported an average mixing ratio in the northern hemisphere of 11 to 17 ppt, with the average in the southern hemisphere of less than 3 ppt. Over the Pacific Northwest, an average tropospheric mixing ratio of 20 ± 3.9 ppt was found (Cronn et al., 1977a). Harsch (1977) reported levels

TABLE 3-7. AMBIENT AIR-MIXING RATIOS OF TRICHLOROETHYLENE

Location	Date of measurements	Mixing ratio (ppb) Maximum	Minimum	Average	Reference
ARIZONA					
Phoenix	04/23-05/06 1979	3.0696	0.0117	0.4835 ± .5869	Singh et al., 1981
Grand Canyon*	11/28-12/05 1977	0	0	0	Pellizzari, 1979a
ARKANSAS					
Helena	11/30 1976	≤ 0.03	≤ 0.03	≤ 0.03	Battelle, 1977
El Dorado*	06/13-07/08 1978	13	0.13	3.1 ± 3.8	Pellizzari, 1979a
CALIFORNIA					
Stanford Hills	11/23-11/30 1975	3.09	0.006	0.093 ± 0.32	Singh et al., 1979a
Los Angeles	04/28-05/04 1976	1.77	0.026	0.315 ± .310	Singh et al., 1979a
Los Angeles	04/09-04/21 1979	1.702	0.363	0.399 ± .302	Singh et al., 1981
Point Reyes	12/01-12/12 1975	0.064	0	0.024 ± 0.014	Singh et al., 1979
Palm Springs	05/05-05/11 1976	0.446	0.006	0.035 ± 0.050	Singh et al., 1979
Yosemite	05/12-05/18	0.022	0.008	0.015 ± 0.003	Singh et al., 1979
Mill Valley	01/11-01-27 1977	0.090	0.009	0.026 ± 0.094	Singh et al., 1979
Riverside	04/25-05/04 1977	0.476	0.057	0.266 ± 0.094	Singh et al., 1979
Menlo Park					Singh et al., 1979
Upland	08/13-09/23 1977	0.64	0	0.080 ± 0.22	Pellizzari, 1979a
Badger Pass	05/05-05/13 1977	0.016	0.001	0.012 ± 0.002	Pellizzari, 1979a

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	Minimum	Average	Reference
Point Arena	05/23-05/30 1977	0.012	0.010	0.011 ± 0.001	Pellizzari, 1979a
Point Arena	08/30-09/05 1978	0.034	0.008	0.017 ± 0.007	Pellizzari, 1979a
San Jose	08/21-08/27 1978	2.192	0.048	0.417 ± 0.425	Pellizzari, 1979a
Oakland	06/28-07/10 1979	1.5582	0.0141	0.1876 ± 0.2697	Singh et al., 1981
La Jolla*		3.9	0	1.3 ± 1.2	Su and Goldberg, 1976
DELAWARE					
Delaware City	07/08-07/10 1974	0.56	< 0.05	0.35	Lillian et al., 1975
LOUISIANA					
Baton Rouge*	11/18 1976 05/20 1977	0.65	0	0.4 ± 0.23	Pellizzari et al., 1979; Battelle, 1977
Lake Charles	12/06 1976 06/26 1978	2.1	0.073	1.6 ± 0.79	Battelle, 1977; Pellizzari, 1979b
MARYLAND					
Baltimore	07/11-07/12 1974	< 0.05	< 0.05	-	Pellizzari et al., 1979
NEW JERSEY					
Rutherford*	05/01 1978 12/29 1979	8.6	0	1.15	Bozzelli and Kebbekus, 1983
Somerset *	07/18-07/26 1978	0.28	0	0.17 ± 0.17	Bozzelli and Kebbekus, 1979
South Amboy	01/27 1979 12/29 1979	8.5	0	0.08 ± 0.28 0.4 ± 1.4	Bozzelli and Kebbekus, 1983

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	Minimum	Average	Reference
Batso	02/26 1979 12/29 1979	1.3	0	0.08 ± 0.28	Bozzelli and Kebbekus, 1983
Seagirt	06/18-06/19 1974	2.8	< 0.05	0.26	Lillian et al., 1975
Newark	03/23 1976 12/29 1979	1.9	0	0.25	Bozzelli and Kebbekus, 1983
Sandy Hook	07/02-07/05 1974	0.8	< 0.05	0.34	Lillian et al., 1975
Elizabeth	09/15 1978 12/29 1979	6.4	0	0.76	Bozzelli and Kebbekus, 1983
Fords*	03/26 1976 09/27 1978	3.1	0	1.9 ± 1.1	Pellizzari, 1977; Pellizzari et al., 1979
Middlesex*	07/23-07/28 1978	0.36	0	0.22 ± 0.11	Bozzelli and Kebbekus, 1979
Edison*	03/24 1976 09/24 1978	6.1	0.54	2.7 ± 2.1	Pellizzari, 1978; Pellizzari, 1979; Pellizzari, 1977b
Carlstadt*	09/28-09/30 1978	9	3	6.4 ± 3.1	Pellizzari et al., 1979
Camden	04/03-10/24 1979	3.5	0	0.42 ± 0.87	Bozzelli and Kebbekus, 1983
Bayonne	March-Dec. 1973	8.8	< 0.05	0.92	Bozzelli and Kebbekus, 1983
Boundbrook*	03/26 1976 09/20 1978	4.1	0	2.4 ± 1.8	Pellizzari, 1977; Pellizzari et al., 1979
Bridgewater*	07/17 1978 08/05 1978	6.9	0	0.35 ± 0.29	Bozzelli and Kebbekus, 1979
Rahway*	09/20-09/22 1978	18	3.4	11 ± 7.8	Pellizzari et al., 1979

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb)			Reference
		Maximum	Minimum	Average	
NEW YORK					
NYC	06/27-06/28 1974	1.1	0.11	0.71	Lillian et al., 1975
Whiteface Mtns.	09/16-09/19 1974	0.35	< 0.05	0.10	Lillian et al., 1975
Niagara Falls/Buffalo	Fall, 1978	0.11	0.05	0.10	Pellizzari et al., 1979
OHIO					
Wilmington	07/16-07/26 1974	0.63	< 0.05	0.19	Pellizzari et al., 1979
NEVADA					
Reese River	05/14-05/20 1977	0.016	0.001	0.012 ± 0.002	Singh et al., 1979
KANSAS					
Jetmar	06/01-06/07 1978	0.021	0.007	0.013 ± 0.004	Singh et al., 1979
WASHINGTON					
Pullman	December 1974-February 1975	-	-	< 0.005	Grimsrud and Rasmussen, 1975
Pacific Northwest	March 1976	-	-	0.020 ± 0.0039	Cronn et al., 1977a
PACIFIC OCEAN					
(lat 37°N)	April 1977	-	-	0.0124	Cronn et al., 1977b
PANAMA CANAL ZONE					
(lat 9°N)	July 1977	-	-	0.015	Cronn and Robinson, 1978

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	Minimum	Average	Reference
NORTH ATLANTIC OCEAN	October 1973	-	-	< 0.005 ± 0.0657	Lovelock, 1974a
WESTERN IRELAND	June-July 1974	-	-	0.015 ± 0.0121	Lovelock, 1974a
ENGLAND	March 1972	.005	4 x 10^{-4}	.002	Murray and Riley, 1973a
JAPAN					
Tokyo	09/10-10/27 1975	47.1	0.8	3.5 ± 3.7	Tada et al., 1976
Various sites	Summer, 1979	0.038	0.005	0.014 ± 0.010	Makide, et al., 1980
WESTERN EUROPE	1976	8.5	< 0.02		Correia et al., 1977
COLORADO					
Denver*	06/16-06/26 1980	0.41	0.028	0.2 ± 0.11	Singh et al., 1980
MISSOURI					
St. Louis*	05/30 1980 06/08 1980	0.24	0.019	0.11 ± 0.063	Singh et al., 1980
TEXAS					
Aldine	06/22 1977 10/20 1977	0.029	0	0.009 ± 0.017	Pellizzari et al., 1979
El Paso	04/05 1978 05/01 1978	0.3	0	0.15 ± 0.11	Pellizzari, 1979a
Freeport	08/09 1976 11/10 1976	0.029	0	0.001 ± 0.004	Battelle, 1977b; Pellizzari et al., 1979
Houston	07/27 1976 05/24/1980	0.33	0	0.12 ± 0.098	Pellizzari et al., 1979; Singh et al., 1980

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	 Minimum	 Average	Reference
WASHINGTON					
Auburn*	01/10-01/11 1977	0.83	0.19	0.64 ± 0.29	Battelle, 1977b
WEST VIRGINIA*					
Nitro	09/27 1977 11/18 1977	0.067	0	0.032 ± 0.035	Pellizzari, 1978
West Belle	09/27 1977 11/18 1977	0	0	0	Pellizzari, 1978
VIRGINIA*					
Front Royale	09/29 1977 11/16 1977	0.009	0	0.003 ± 0.004	Pellizzari, 1978

*Data obtained from summary report of Brodzinsky and Singh, 1982.

between 22 ppb and 8.7 ppb in more than 20 different indoor environments. Brodzinsky and Singh (1982), in their review of monitoring data, reported that background concentrations average about 30 ppt. A maximum level of 87 ppb was reported for Newark, New Jersey.

3.3.3.2 Water. Trichloroethylene has been detected in a number of raw water sources, finished drinking waters, and subsurface waters in the United States. The occurrence of TCI in waters of the United States has been reviewed by the U.S. Environmental Protection Agency (1979).

3.3.3.2.1 Drinking water. Dowty et al. (1975) detected TCI in finished drinking water in the New Orleans area. It was reported to pass through the water treatment plant without alteration. It was also reported to be incompletely removed after passage of water through a charcoal filtering unit. Detection was made by GC-MS.

In a comprehensive surface water monitoring effort for TCI, Ewing et al. (1977) have shown TCI to be a common low-level (ppb) contaminant of surface waters in the vicinity of urban/industrial areas. TCI was reported at concentrations of 1 μg/L (ppb) or greater in 72 of 179 surface water samples from U.S. urban/industrial areas from 23 states (Table 3-8). Ewing also reported samples of drinking water which were not included in this total.

A concentration of 8.5 ppb was found in the drinking water of the town of Grand Island, New York (Coleman et al., 1976) using headspace GC-EC. In 10 lake water samples from this area, an average concentration of 11.9 ppb ± 2.5 percent was found. Coleman et al. (1976) detected TCI in the drinking water of four cities in 1975. The concentrations were: 0.1 ppb (Cincinnati); 0.3 ppb (Miami); <0.1 ppb (Ottumwa, Iowa); and 0.5 ppb (Philadelphia). No TCI was detected in drinking water samples for Seattle. Identification and quantification were made by GC-MS using the purge/trap technique.

As evidenced by the differences reported in the individual studies above, the frequency of contamination and the amount of TCI found in potable waters depend in part on the source of water being monitored.

The surface water supplies of 133 cities using surface water supplies have been sampled during federal surveys. Thirty-two percent of the finished waters serving these cities were found to contain TCI. The concentration of TCI in these water supplies ranged from 0.06 to 3.2 μg/L. The average concentration in positive water supplies was 0.47 μg/L. Thirty percent of all supplies contained a level of TCI which was below 1 μg/L. The remaining two percent of the supplies contained a concentration between 1 to 4 μg/L.

TABLE 3-8. MEAN LEVELS OF TCI IN SURFACE WATERS[a]

	Surface water samples			Mean levels (µg/L)	
State	Positive for TCI	Total	Percent positive for TCI	All samples[b,c]	Positive samples only
Alabama	1	7	14	0.5	1.0
Delaware	4	10	40	1.2	2.3
Illinois	17	25	68	0.6	3.2
Indiana	1	4	25		1.0
Kentucky	1	3	33	0.7	1.0
Louisiana	1	7	14	0.6	1.0
Michigan	1	8	13	23.9	188
Minnesota	2	9	22	1.9	7
New Jersey	14	15	93	4.0	4.3
New York	10	20	50	1.6	2.6
Pennsylvania	14	21	67	2.3	3.2
Tennessee	1	6	17	0.9	3.0
Texas	4	8	50	1.2	1.8
West Virginia	1	3	3	0.7	1.0
All Sites	72	179	40	2.7	5.9

[a] Source: Ewing et al. (1977); drinking water samples not reported in these data.

[b] Mean levels were calculated by substituting a value of one-half the reporting limit of 1 ppb (1 µg/L) for samples reported as having non-detectable (i.e., less than 1 µg/L).

[c] Levels of TCI in sample from the following states (number of sites per state in parentheses) were reported as not detectable (i.e., less than 1 µg/L):

California (9)
Iowa (2)
Mississippi (1)
Missouri (3)
North Carolina (1)
Oregon (3)
Washington (2)
Wisconsin (6)

In 25 cities that draw their water from underground sources, water supplies were sampled for TCI during the NOMS, NORS, SRI and EPA Region V surveys. Thirty-six percent of these finished waters contained detectable concentrations of this chemical. The median level detected was 0.31 μg/L with a range from 0.11 to 53.0 μg/L.

The Community Water Supply Survey examined an additional 330 ground water systems selected randomly from across the United States. Four percent of these systems contained a detectable level of TCI. Seventy percent of these detectable levels of TCI were between 0.5 to 5 μg/L. TCI was found in finished water from small and large systems alike.

In analyses of drinking waters from sites near producer and user facilities and from a background site, Battelle (1977) found the following concentrations in 1977: 19 ppb (Freeport, Texas); 0.4 ppb (Baton Rouge, Louisiana); 0.1 ppb (Lake Charles, Louisiana); 32 ppb (Des Moines, Iowa); and 22 ppb (Helena, Arkansas).

Boateng et al. (1984) have described the geology, hydrology, and chemistry of both shallow and deep aquifers in relation to the occurrence of TCI in two production wells.

3.3.3.2.2 Rivers, treatment plant effluents. Up to 403 ppb TCI was detected by Battelle in surface waters near specific manufacturing facilities (1977). Trichloroethylene was detected in both industrial wastewater and river water receiving the waste (Jungclaus et al., 1978).

Murray and Riley (1973a) detected TCI in surface sea water in the eastern Atlantic ocean.

Bellar et al. (1974) found an increased concentration of TCI after chlorination treatment of the effluent from a sewage-treatment facility. Before chlorination, the concentration detected by GC-MS was 8.6 ppb; after chlorination, the concentration increased to 9.6 ppb.

Case reports describing TCI contamination of well waters and water supplies have been reviewed elsewhere (U.S. EPA, 1979). Concentrations as high as 330 ppb have been reported.

Correia et al. (1977) measured TCI in river, canal, and sea waters that receive effluent from production and user sites. Values ranged from 0.02 to 74 μg/L (ppb w/w), showing the effect of these additions on background levels. Dilution effects and losses to the surrounding air were noted.

3.3.3.3 Soil and Sediment. Battelle (1977) reported the presence of TCI in soil samples in the vicinity of producers and users at levels up to 5.6 ppb. In freshwater sediment taken near the Hooker Chemical facility at Taft, Louisiana, up to 300 ppb TCI was detected (Battelle, 1977). Trichloroethylene was detected in the soil in the Love Canal area in Niagara Falls, New York (U.S. EPA, 1979).

Pearson and McConnell (1975) found up to 9.9 ppb in sediment from Liverpool Bay, England. Murray and Riley (1973b) found TCI in river mud.

Rogers and McFarlane (1981) reported that aluminum (Al^{+3})-saturated montmorillonite clay sorbed about 17 percent of the TCI applied (100, 500, and 1,000 ppb). The organic carbon content was assumed to be zero. There was no apparent absorption when calcium-saturated montmorillonite clay was used. With two silty clay loams (organic carbon content ≅ 2 percent), less than 6 percent of the TCI available was sorbed. When soil sorption was normalized, based on the soil organic carbon contents, a correlation was found between water solubility, sorption constant, and the partitioning coefficient.

3.4 ECOLOGICAL EFFECTS

Trichloroethylene has been tested for acute toxicity in a limited number of aquatic species. This section presents observed levels reported to result in adverse effects under laboratory conditions. Such parameters of toxicity are not easily extrapolated to environmental situations. Test populations themselves may not be representative of the entire species in which susceptibility of various lifestages to the test substance may vary considerably.

Guidelines for the utilization of these data in the development of criteria levels for TCI in water are discussed elsewhere (U.S. EPA, 1980).

The toxicity of TCI to fish and other aquatic organisms has been gauged principally by flow-through and static testing methods (Committee on Methods for Toxicity with Aquatic Organisms, 1975). The flow-through method exposes the organism(s) continuously to a constant concentration of TCI while oxygen is continuously replenished and waste products are removed. A static test, on the other hand, exposes the organism(s) to the added initial concentration only. Both types of tests are commonly used as initial indications of the potential of substances to cause adverse effects.

Alexander et al. (1978) used both flow-through and static methods to investigate the acute toxicity of several chlorinated solvents, including TCI, to adult fathead minnows (Pimephales promelas). Studies were conducted in accordance with test methods described by the U.S. Environmental Protection Agency (1975). The lethal concentration of each solvent tested to produce 50 percent mortality (LC_{50}) was lower in the flow-through than in the static-type experiment. The flow-through method was considered the better of the two for volatile solvents such as TCI. The 96-hr LC50 concentration for TCI was 40.7 mg/L in the flow-through test. The 95 percent confidence band was between 31.4 and 71.8 mg/L. Of four solvents tested (perchloroethylene, methylene chloride, methyl chloroform, and TCI), TCI had the second highest toxicity (after perchloroethylene).

In acute static experiments with bluegills, TCI was reported to have a 96-h LC_{50} value of 44.7 mg/L (U.S. EPA, 1978b). With Daphnia magna as the test organism in a 48-hr static experiment, the LC_{50} for TCI was 85.2 mg/L. In a chronic test with this organism, no adverse effects were found at the highest test concentrations of 10 mg/L.

Lay et al. (1984) evaluated the effect of TCI on Daphnia magna and phytoplankton species in a natural pond. The pond was compartmentalized and two compartments each were used for a single high dose of 110 ng/L, a single low dose of 25 ng/L, and controls. Complete elimination of Daphnia magna in the high dose groups was noted at 24 and 72 hours after dosing. In the low dosage groups, TCI was less toxic to Daphnia. However, a significant decrease in population was noted in both treated compartments from day 3 to day 28 of the experiment. The relative abundance and absolute number of all phytoplankton individuals counted increased with the increase of TCI concentrations.

Investigation of the bioconcentration potential of TCI for bluegill indicated that the bioconcentration factor (BCF) was 17. The half-life of this compound in tissues was less than 1 day (U.S. EPA, 1980; U.S. EPA, 1978b). These data suggest that residues of TCI at exposure concentrations below those directly toxic to aquatic life are not likely (U.S. EPA, 1980).

Few data are available relating to effects of TCI on saltwater aquatic life. Borthwick (1977) exposed sheepshead minnows and grass shrimp to 20 and 2 mg/L, respectively, and noted erratic swimming, uncontrolled movement, and loss of equilibrium after several minutes.

3.5 CRITERIA, STANDARDS, AND REGULATIONS

The current occupational standard for TCI established by the Occupational Safety and Health Administration in 1972 (General Industry Standards, OSHA, 1978) is 100 ppm (525 mg/m^3) as a time-weighted average (TWA) over an 8-hr period. More recently, the National Institute for Occupational Safety and Health (NIOSH) (Page and Arthur, 1978) has noted that the current standard appears to be inadequate for protection of workers from the carcinogenic potential of TCI. A level of 25 ppm, as a TWA, was the recommended standard proposed by NIOSH, based on engineering control capabilities. The American Council of Governmental Industrial Hygienists has established a TLV® of 50 ppm.

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4. PHARMACOKINETICS AND METABOLISM

4.1 ABSORPTION AND DISTRIBUTION

Trichloroethylene is a highly volatile liquid whose vapors exert pharmacologic effects common to general inhalation anesthetics. Although it is no longer widely used as an anesthetic or analgesic agent, the highly effective fat-solvent properties of TCI are the basis of its numerous industrial uses. At work sites where this compound is manufactured or used, the principal mode of entry into the body is inhalation and lung absorption of TCI vapor. Dermal absorption occurs with direct liquid contact in some degreasing applications and consumer uses (e.g., paint stripping). Oral absorption is also an important route of absorption. Although sparingly soluble in water (1.0 gm/ℓ, 25°C), TCI is present in the water supplies of many of our cities and in their atmospheres, and it has been identified in measurable amounts in the food chain. Hence, ordinary sources in the nonindustrial environment include air, food, and drinking water. Indeed, TCI has been detected in notable amounts in the breath of healthy humans and in postmortem human tissue samples (Conkle et al., 1975; McConnell et al., 1975; Barkley et al., 1980).

4.1.1 Dermal Absorption

Skin absorption from TCI vapor exposure is negligible, although direct skin contact with the liquid by immersion of the hands, or partial body immersion, may result in significant absorption. Uptake through the skin is related to the type of skin exposed and the area of skin exposure as well as to the duration of skin exposure. Tsuruta (1978) estimated skin absorption of TCI in mice by *in vivo* and *in vitro* techniques. The percutaneous absorption rate of TCI in mice was reported to be between 59.8 and 92.4 $\mu mol/min/cm^2$ or between 7.82 and 12.1 $\mu g/min/cm^2$. From this study, Tsuruta concluded that direct contact with TCI, even if the percutaneous absorption rate of TCI is different between human skin and mouse skin, may have the potential to contribute importantly to the overall exposure to TCI. However, Stewart and Dodd (1964) have experimentally estimated skin absorption of TCI in volunteers by noting its appearance and concentration in lung alveolar air after thumb immersion. The mean peak concentration of TCI in expired air 30 minutes after immersion was only 0.5 ppm,indicating that the rate of absorption through the skin is very slow, even after allowing for the possibility of lipid storage and for metabolism to urinary metabolites (see below). In addition, Sato and Nakajima (1978) demonstrated that for skin exposure in man, a large proportion of absorbed TCI is eliminated unchanged through the lungs. These authors also concluded that TCI would rarely be absorbed through the human skin in toxic amounts during normal industrial use.

Recently Jakobson et al. (1982) carried out dermal absorption studies on TCI with guinea pigs. Liquid contact (skin area, 3.1 cm^2) was maintained for up to 6 hours and solvent concentration was monitored in blood during and following dermal applications. Blood concentration (reflecting absorption rate) increased rapidly, peaking at 0.5 hour (0.8 μg/ml blood), and then decreased despite continuing exposure (0.46 μg/ml blood after 6 hours). This pattern was characteristic of halogenated hydrocarbon solvents of low water solubility. Of 12 solvents tested, TCI produced the second lowest blood concentrations from dermal absorption.

4.1.2 Oral Absorption

TCI is an uncharged, nonpolar, and highly lipophilic compound (Table 4-1) and consequently can be expected to readily cross the gastrointestinal mucosal barrier by passive diffusion. In man, absorption through the gastrointestinal mucosa is extensive, as documented by the numerous cases of poisoning by oral ingestion reported over the years (Defalque, 1961; Vyskocil and Polak, 1963; Wiecko, 1966; Vignoli et al., 1970; Migdal et al., 1971). In animals, the oral LD_{50} in mice, rats and dogs are similar, being 3.2, 4.9 and 2.8 g/kg, respectively (Klaasen and Plaa, 1966, 1967; Smyth et al., 1969).

Daniel (1963) dosed rats by stomach-tube with ^{36}Cl-labeled TCI liquid (40 to 60 mg/kg b.w.) and recovered 90 to 95 percent of the label in expired air and urine, suggesting virtually complete absorption by the route. Similar results have been reported recently by Dekant et al. (1984) and Prout et al. (1984). Dekant et al. dosed by gavage both rats and mice with 200 mg ^{14}C-TCI/kg in corn oil vehicle and recovered 93 to 98 percent of the radiolabel in expired air and urine (Table 4-1). Prout et al. administered ^{14}C-TCI in corn oil vehicle as single intragastric doses of 10, 500, 100, and 2000 mg/kg to both rats and mice and recovered 91 to 98 percent of the doses in expired air and urine (Table 4-2). Peak blood levels occurred at about 1 hour in mice and 3 hours in rats, indicating rapid absorption from the GI tract.

TABLE 4-1. RECOVERY OF RADIOACTIVITY FOR 72 HOURS AFTER A SINGLE ORAL DOSE OF ^{14}C-TCI (200 mg/kg) TO FEMALE WISTAR RATS AND NMRI MICE

	Time (hr)	As % Recovered Radioactivity*	
		Rats	Mice
Exhaled air			
Unchanged, ^{14}C-TCI	0 - 72	52.0	11.0
$^{14}CO_2$	0 - 72	1.9	6.0
		53.9	17.0
Urine	0 - 12	22.0	30.0
	12 - 24	17.0	39.0
	24 - 48	2.0	6.0
	48 - 72	0.2	1.0
Cage rinse		0.2	0.1
		41.4	76.3
Feces	0 - 72	1.8	4.9
Carcass, tissues	0 - 72	2.9	2.0

*N = 2 rats and 3 mice; total recovered radioactivity was 93 to 98% of the administered radioactivity given the rats and mice. The average weight of the rats was 240 gm and the mice 25.5 gm.

Source: DeKant et al. (1984).

Withey et al. (1983) demonstrated in rats that gastrointestinal absorption of TCI is considerably more rapid in water solution than when given in corn oil as a vehicle. Following an 18 mg/kg intragastric dose in 5 ml water or corn oil to fasting rats (400 g), the peak blood concentration (5.6 minutes for aqueous solution) averaged 15 times higher for water than for corn oil solution (14.7 versus 1.0 µg/ml); however, the peak blood concentration was reached faster for water solution than for oil solution, which exhibited a second delayed peak 80 minutes post absorption. Comparison of the area under the blood concentration curve (AUC) during the absorptive phase reflects the rate and extent of absorption. The ratio of the AUCs for 5 hours after dosing was 218, water:corn oil. These results illustrate the effect of solution

TABLE 4-2. DISPOSITION OF SINGLE DOSES OF ^{14}C-TCI ADMINISTERED BY GAVAGE TO OSBORNE-MENDEL RATS AND B6C3F1 MICE

Dose, mg/kg	% Dose Excreted **						
	Unchanged ^{14}C-TCI	Metabolized					Recovery
		$^{14}CO_2$	Urine	Feces	Carcass	Total	
Osborne-Mendel Rats*							
10	1.3	10.2	69.4	8.3	8.4	96.3	97.6
500	42.7	3.7	43.6	4.3	3.8	55.4	98.1
1000	56.2	2.9	31.1	3.1	2.4	39.5	95.7
2000	77.8	1.4	14.0	3.4	1.3	20.1	97.9
B6C3F1 Mice*							
10	3.9	11.7	59.4	17.2	4.8	93.1	97.0
500	6.0	8.9	63.7	14.5	4.4	91.5	97.5
1000	17.5	7.6	46.4	15.0	8.6	77.6	95.1
2000	13.6	7.8	47.5	19.2	3.8	78.3	91.9

*Means of groups of 4 animals; rat weights 180 to 200 gm and mouse 25 to 32 gm.

**During 72-hr period after dosing.

Source: Prout et al. (1984).

medium on TCI intestinal absorption; the much slower absorption of TCI dissolved in corn oil is probably due to the high lipid solubility (and poor aqueous solubility) of this compound which remains as a depot in the oil. Hence, absorption is limited to the rate of absorption of the corn oil itself, or the corn oil effectively acts as a slow-release dosage form.

4.1.3 Pulmonary Absorption

4.1.3.1 Man. Inhaled TCI rapidly equilibrates across the lung alveolar endothelium. The blood/gas partition coefficient of 9.92 at 37°C is comparable to the anesthetic gases--chloroform, diethylether, and methoxyfluorane--but its lipid solubility is considerably higher (olive oil/gas coefficient, 960 at 37°C) (Powell, 1947; Eger and Larson, 1964). Thus, pulmonary uptake of TCI is rapid and extensive with considerable distribution into lipid of body tissues (Table 4-3).

TABLE 4-3. PULMONARY UPTAKE OF TCI FOR 25 VOLUNTEERS EXPOSED TO TCI FOR FOUR CONSECUTIVE 30-MIN PERIODS AT REST AND DURING EXERCISE (50 TO 150 WATTS)

Exposure concentration, mg/m^3	Conditions	Amount inhaled, mg	Amount taken up,* mg
540	rest	149 ± 6	79 ± 4
1080	rest	313 ± 15	156 ± 9
540	Exercise (50w)	395 ± 6	160 ± 5
1080	Exercise (50w)	803 ± 12	308 ± 16

540 and 1080 $mg/m^3 \equiv$ 100 and 200 ppm, respectively.

*Calculated from difference in inspired air and expired air x ventilation volume x time. Retention value averaged = 45 percent.

Source: Astrand and Ovrum (1976).

Pulmonary uptake is proportional to the magnitude of the gas partial pressure difference between alveoli and pulmonary venous blood. This difference diminishes when all body tissues achieve equilibrium with the alveolar (arterial) partial pressure. Initially, uptake is rapid, but about 8 hours of exposure is required for complete tissue equilibrium to be achieved (Fernandez et al., 1977; Sato et al.; 1977; Monster et al., 1976, 1979; Muller et al., 1974). Figure 4-1 shows that equilibrium is achieved most rapidly (4 to 6 hours) with the rich blood-perfused tissues. The poorly perfused fat tissues (FG) require longer than 8 hours for equilibrium.

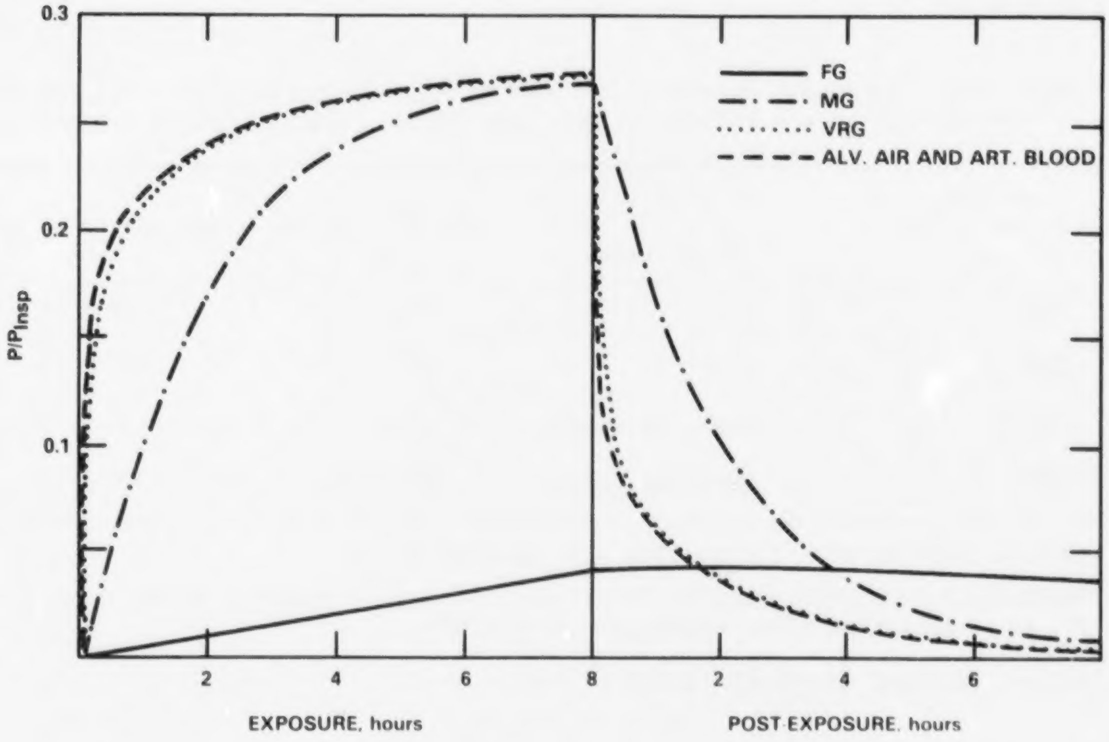


Figure 4-1. Predicted partial pressure of TCl in alveolar air and tissue groups during and after an 8 hour exposure of 100 ppm.

Source: Fernandez et al. (1977).

At equilibrium with a given concentration of TCI in inspired air, the difference between the inspired air concentration of TCI and alveolar concentration of TCI provides a measure of gas uptake. With appropriate accounting for ventilation (about 4 to 8 L/min at rest) and duration of exposure, the amount taken up by the body, or retention (dose; body burden), can be calculated. At alveolar and tissue equilibrium, the retention reflects disposition within the body by tissue storage, metabolism, or excretion by routes other than the lungs. Retention of 36 to 75 percent has been reported for TCI by various investigators (Soucek and Vlachova, 1960; Bartonicek, 1962; Nomiyama and Nomiyama, 1971, 1974a,b), with higher values (70 to 75 percent) reported from more recent studies (Fernandez et al., 1975; Monster et al., 1976, 1979) (Tables 4-3 and 4-4). The retention value is independent of the inspired TCI concentration. In contrast, the amount (dose) of TCI absorbed into the body is directly proportional to the TCI concentration in the inspired air (Fernandez et al., 1975; Monster et al., 1976, 1979; Muller et al., 1974). The body burden of TCI also increases with duration of exposure, repeated daily exposures and exercise (increased ventilation) at a given inhaled air concentration (Astrand and Ovrum, 1976; Monster et al., 1976, 1979). These relationships between inspired air concentration, pulmonary uptake and body burden are illustrated by the data of Astrand and Ovrum and of Monster et al. in Tables 4-3 and 4-4. Astrand and Gamberale (1978) exposed volunteers to 100 and 200 ppm (ventilation volume 9.3 L/min) for 70 minutes and observed a linear relationship between pulmonary uptake (Q) and alveolar concentration (A) divided by inspired air concentration (I)

$$Q = 0.72 \frac{A}{I} + 74.91.$$

However, as about eight or more hours are required for complete body equilibration, this relationship only approximates steady-state inhalation conditions.

The blood concentration of TCI during inhalation and in the elimination phase after exposure closely parallels alveolar gas concentration (Figure 4-1 and Figure 4-2) (Muller et al., 1974; Monster et al., 1976, 1979; Stewart et al., 1974; Sato et al., 1977; Fernandez et al., 1977). The kinetics of tissue uptake, accumulation, and equilibration of TCI is a function of blood concentration, the blood flow/tissue mass, and the relative solubilities of TCI in tissues (Table 4-5). Therefore, TCI most rapidly attains equilibrium

TABLE 4-4. PULMONARY UPTAKE OF TCI FOR 4 SUBJECTS AT REST EXPOSED TO 70 AND 140 PPM TCI FOR 4-HOUR PERIODS AND 3-HOUR PERIODS, PLUS TWO 30-MIN PERIODS OF WORK (100 WATTS)

Exposure concentration and conditions	Dose, mg Subject				Mean mg
	A	B	C	D	
70 ppm, rest	320	430	330	470	390
70 ppm, rest + work	500	450	470	660	520
140 ppm, rest	740	790	710	790	755
140 ppm, rest + work	1050	1100	970	1100	1055

Retention value average ≡ 38 percent.

Source: Monster (1976).

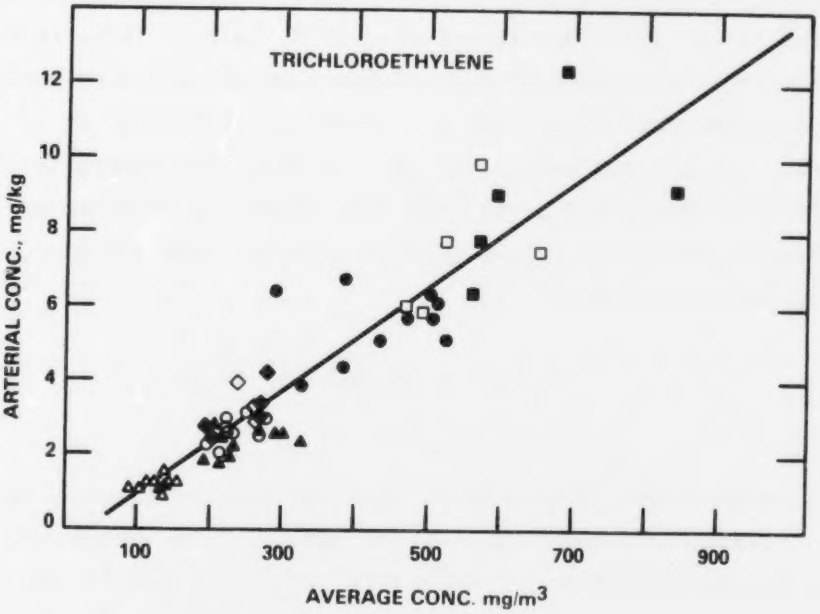


Figure 4-2. The relationship between the concentration of TCI in arterial blood and alveolar air at the end of inhalation exposures (30 min periods, for 15 male subjects). Symbols stand for one exposure period for a subject at rest, and at various levels of exercise. The regression line is $y = -0.489 + 0.014\ X$.

Source: Astrand and Ovrum (1976).

TABLE 4-5. PARTITION COEFFICIENTS OF TCI FOR VARIOUS BODY TISSUES AND COMPONENTS OF RAT AND MAN

Rat	Mean values
Blood/air	25.82
Lung/blood	1.03
Heart/blood	1.10
Kidney/blood	1.55
Liver/blood	1.69
Muscle/blood	0.63
Brain/blood	1.29
Testes/blood	0.71
Spleen/blood	1.15
Fat/blood	25.59
Man	
Blood/air	9.92
Fat/air	674.4
Lecithin/air	387.9
Cholesterol/air	52.15
Cholesterol oleate/air	261.93
Triolein/air	848.24

Conversion factors:

1 ppm vapor in air = 5.38 mg/m^3 at 25°C, 760 torr.

Source: Adapted from Sato et al. (1977).

by passive diffusion into the vessel-rich group (VRG) of tissues (brain, heart, kidneys, liver, and endocrine and digestive systems), more slowly with the lean mass (MG) (muscle and skin), and last with adipose tissue, i.e., fat groups (FG). As determined from elimination kinetics following exposure, TCI distributes from blood into these three major compartments at approximate rate constants of 17 hr^{-1} ($t_{\frac{1}{2}}$, 2.4 min) for VRG, 1.7 hr^{-1} ($t_{\frac{1}{2}}$, 25 min) for MG, and 0.2 hr^{-1} ($t_{\frac{1}{2}}$, 3.4 hr) for FG (Fernandez et al., 1975, 1977; Sato et al., 1977). Though the lean muscle mass is 50 percent of the body volume and adipose tissue is 20 percent, saturation and desaturation proceed more rapidly from the MG compartment ($t_{\frac{1}{2}}$, 25 min) than from the FG compartment ($t_{\frac{1}{2}}$, 3.4 hr) because of the considerably greater solubility of TCI in lipid (Table 4-1). Interperson variations in TCI uptake (dose) are therefore influenced primarily by lean body mass and secondarily by adipose tissue mass (Monster et al., 1976, 1979).

4.1.3.2 Animals. Stott et al. (1982) have determined the pulmonary uptake of TCI in mice and rats exposed to 10 and 600 ppm (54 and 3228 mg/m^3). The animals were exposed in a 30-L glass inhalation chamber to ^{14}C-TCI, the concentration of which was maintained within 10 percent of target. At termination of exposure (6 hours), the cumulative pulmonary uptake was estimated by determining the radioactivity of carcass, and of TCI and metabolites in expired air, urine and feces. The data for mice and rats are given in Tables 4-6 and 4-7, respectively. For mice, the body burdens for 6-hr exposure to 10 and 600 ppm (54 and 3228 mg/m^3) were 78.5 and 3138 µmol equivalents/kg (10.31 and 412.3 mg/kg; or 0.24 and 9.48 mg/mouse), respectively. For rats, amounts were 35.8 and 1075 µmol equivalents/kg (4.7 and 141.3 mg/kg; or 1.03 and 31.09 mg/rat), respectively. For these species the 6-hr pulmonary uptake was not directly proportional to inspired air concentration level, as observed in man. Furthermore, total uptake of TCI, rat versus mouse, is more closely related to their relative body surface areas--$(220/23)^{2/3}$ = 4.5-fold--than it is to their relative body weights, 9.6-fold. Presumably, this difference is primarily related to the overriding influence of metabolism of TCI on uptake rather than to differences in other factors such as ventilation, minute volume, and body weight.

4.1.4 Tissue Distribution and Concentrations

TCI distributes to all body tissues and appears in sweat and saliva (Bartonicek, 1962). It crosses the blood-brain barrier (anesthetic effect) and the placental barrier. Helliwell and Hutton (1950), investigating the distribution in pregnant sheep and goats, found that TCI appeared in the fetal circulation within 2 minutes of exposure; at equilibrium, the fetal/maternal blood concentration ratio was unity. The partition coefficients for various tissues of rat and man are given in Table 4-5 (from Sato et al., 1977).

4.1.4.1 Man. There is little direct analytical information of tissue levels after various exposure concentrations of TCI. Tissue concentrations are expected to be proportional to exposure concentrations and to duration of exposure. Fernandez et al. (1977), from their mathematical model of TCI uptake, distribution, and excretion, based on experimental data, have predicted the partial pressure of TCI in the three major compartmental tissue groups, vessel-rich group (VRG), muscle group (MG), and adipose tissue (FG), during and after an 8-hr inhalation exposure of 100 ppm (538 mg/m^3). Figure 4-2

TABLE 4-6. RECOVERY OF RADIOACTIVITY FOR 50-HOUR POSTINHALATION EXPOSURE OF MALE B6C3F1 MICE TO 10 OR 600 PPM ^{14}C-TCI FOR 6 HOURS[1]

	10 ppm			600 ppm		
	µmol-eq TCI/ kg body weight		%[2]	µmol-eq TCI/ kg body weight		%[2]
Expired						
1,1,2-TCI	0.617	(0.182)	0.79	76.2	(27.3)	2.4
CO_2	7.38	(0.383)	9.4	299	(37.5)	9.5
Urine	58.1	(18.8)	74.0	2278	(505.0)	72.6
Feces	2.92	(0.542)	3.7	115	(24.6)	3.7
Cage wash[3]	1.10	(0.446)	1.4	85.5	(65.5)	2.7
Skin	2.33	(1.13)	3.0	56.3	(11.9)	1.8
Liver	2.40	(0.401)	3.1	72.2	(10.4)	2.3
Kidney	0.310	(0.310)	0.39	11.1	(3.18)	0.35
Carcass	1.50	(0.316)	1.9	146	(57.9)	4.6
Total body burden	78.5	(17.9)		3138	(616.0)	
Total metabolized	77.9	(18.1)	99.2	3062	(592.0)	97.6

[1] Average of four animals (±SD)

[2] Percentage of recovered radioactivity.

[3] Primarily due to urine.

Source: Stott et al., 1982.

TABLE 4-7. RECOVERY OF RADIOACTIVITY FOR 50-HOUR POSTINHALATION EXPOSURE OF MALE OSBORNE-MENDEL RATS TO 10 OR 600 PPM ^{14}C-TCI FOR 6 HOURS[1]

	10 ppm			600 ppm		
	µmol-eq TCI/ kg body weight		%[2]	µmol-eq TCI/ kg body weight		%[2]
Expired						
1,1,2-TCI	0.778	(0.103)	2.1	227	(6.40)	21.1
CO_2	1.71	(0.295)	4.8	31.2	(2.10)	2.9
Urine	22.6	(5.96)	63.1	594	(79.8)	55.3
Feces	2.52	(0.253)	7.0	38.3	(3.47)	3.6
Cage wash[3]	0.357	(0.204)	1.0	15.1	(10.7)	1.4
Skin	3.78	(1.21)	10.6	76.2	(30.3)	7.1
Liver	1.01	(0.085)	2.8	16.3	(1.13)	1.6
Kidney	0.131	(0.009)	0.37	1.72	(0.083)	0.16
Carcass	2.82	(0.898)	7.9	75.1	(27.7)	7.0
Total body burden	35.8	(5.64)		1075	(87.2)	
Total metabolized	35.0	(5.64)	97.8	847	(81.6)	78.9

[1] Average of four animals (±SD)

[2] Percentage of recovered radioactivity.

[3] Primarily due to urine.

Source: Stott et al., 1982.

shows that VRG and MG are nearly always in equilibrium with arterial blood concentration, but the concentration of TCI in FG increases only very slowly and continues to increase beyond the 8-hr exposure. During desaturation, TCI in FG exhibits a long residence (about 40 hours) for disappearance with detectable FG concentrations remaining even after 70 hours. Because of the long residence of TCI in FG, repeated daily exposure (6 hr/day; 100 ppm) results in an accumulated concentration, as TCI from new exposures adds to residual concentration from previous exposures, until steady-state is reached. This accumulation of TCI in FG is shown in Figure 4-3. Adipose tissue levels of TCI reach a steady-state "plateau" (dependent on the inhalation concentration and daily period of exposure) and thereafter remain relatively constant. For a 6-hr/day, 100-ppm (538 mg/m^3) exposure, Fernandez et al. (1977) predict that FG peak plateau concentration occurs 5 to 7 days after initial daily exposure.

4.1.4.2 Rodents. Following intravenous administration of TCI to Wistar rats, Withey and Collins (1980) found a whole-body half-time of disappearance from the rat of 2 to 6 minutes (dependent on dose) but a disappearance half-time of 215 minutes for adipose tissue, indicative of the propensity of TCI to remain in adipose tissue as compared to other tissues. Pfaffenberger et al. (1980), after dosing rats by gavage for 31 days (1 and 10 mg/day), found that blood serum levels of TCI were not detectable (analyzed 2 hours after last dosing); but adipose tissue levels averaged 0.28 and 20.0 µg TCI/g, respectively. Three days after last dosing, 1 µg TCI/g fat still remained in adipose tissues.

Savolainen et al. (1977) analyzed tissue levels of TCI after exposure of rats 6 hours per day for 5 days to 200 ppm (1076 mg/m^3) TCI. Table 4-8 gives the values found. Seventeen hours after exposure on day four, blood and adipose tissue levels were 0.35 and 0.23 mol/g, respectively, indicating long storage in adipose tissue, though other tissues contained virtually no TCI. With exposure on day five, tissue levels in brain, lungs, liver, fat, and blood reached a steady-state within 2 to 3 hours. Partition coefficients were adipose tissue/blood, 10.4; brain/blood, 1.3; lungs/blood, 0.8; and liver/blood, 0.5. The lower value for liver possibly reflects metabolism occurring in this organ, while the high value for adipose tissue reflects high solubility of TCI in lipids.

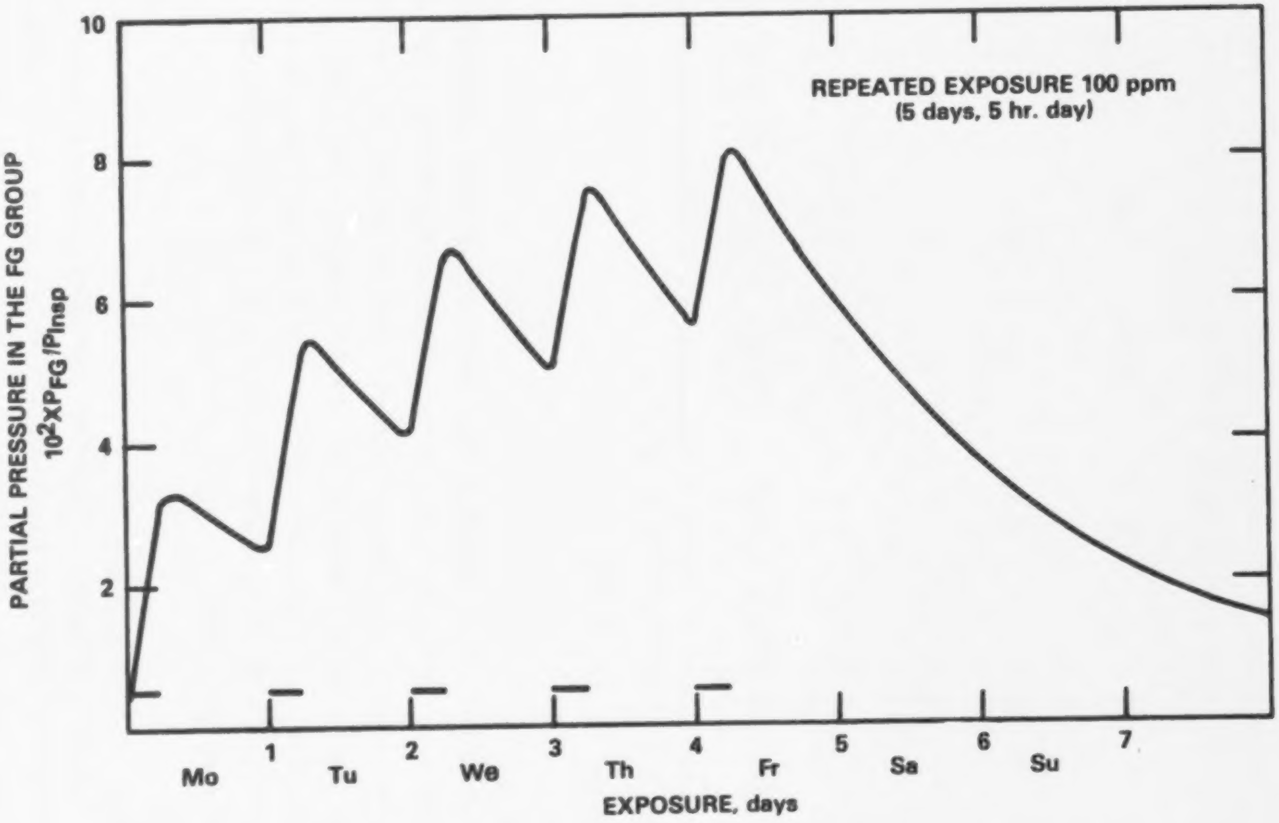


Figure 4-3. Predicted partial pressure of TCI in fatty tissue for a repeated exposure to 100 ppm, 5 days, 6 hours per day.

Source: Fernandez et al. (1977).

TABLE 4-8. ORGAN CONTENTS OF TCI AFTER DAILY INHALATION EXPOSURE OF 200 PPM FOR 6 HOURS PER DAY

Time (hr of exposure) on fifth day	Cerebellum	Cerebrum	Lungs	Liver	Perirenal fat	Blood
			nmol/g			
0 [17 hr after day 4-exposure]	0	0	0.08	0.04	0.23 ± 0.09	0.35 ± 0.1
2	11.7 ± 4.2	9.9 ± 2.7	4.9 ± 0.3	3.6	65.9 ± 1.2	7.5 ± 1.6
3	8.8 ± 2.1	7.3 ± 2.2	5.5 ± 1.4	5.5 ± 1.7	69.3 ± 3.3	6.6 ± 0.2
4	7.6 ± 0.5	7.2 ±1.7	5.8 ± 1.1	2.5 ± 1.4	69.5 ± 6.3	6.0 ± 0.9
6	9.5 ± 2.5	7.4 ± 2.1	5.6 ± 0.5	2.4 ± 0.2	75.4 ± 14.9	6.8 ± 1.2

Values are mean of 2 determinations ± range.

Source: Savolainen et al. (1977).

4.2 EXCRETION

The total elimination of absorbed TCI involves two major processes, pulmonary excretion of unchanged TCI and hepatic biotransformation to urinary metabolites. The details of hepatic biotransformation of TCI are reviewed below. In man, the principal metabolites are trichloroethanol (TCE), TCE-glucuronide, and trichloroacetic acid (TCA). These metabolites are excreted in urine, and in less significant quantities by other excretion routes, such as the bile (Bartonicek, 1962).

4.2.1 Pulmonary Elimination in Man--Pulmonary elimination of unchanged TCI after exposure occurs nearly as an inverted reproduction of its uptake. Alveolar concentration parallels blood concentration; both follow exponential concentration decay curves showing three major components with approximate half-times of 2 to 3 minutes, 30 minutes, and 3.5 to 5 hours (Figures 4-1 and 4-4), usually represented by VRG, MG, and FG compartments, respectively

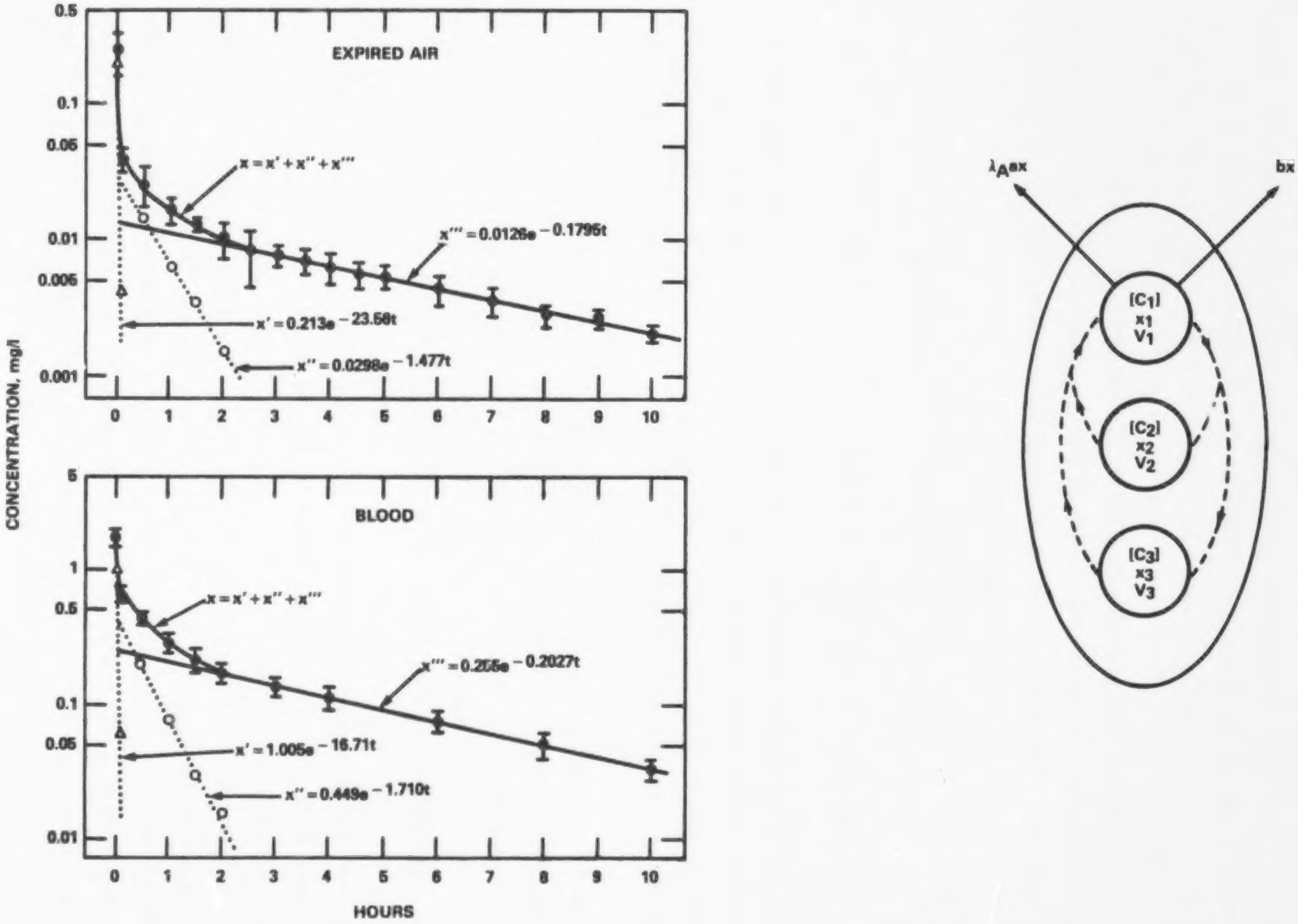


Figure 4-4. Elimination curves of TCI and kinetic model for transfer of TCI in the body. $\lambda\ a\ \chi$ = pulmonary excretion (ventilation x air/blood partition coefficient x blood concentration); bx = metabolic clearance (urine) x blood concentration; V_1, V_2, V_3 volumes of VRG, MG and FG compartments respectively.

Source: Sato et al. (1977).

(Fernandez et al., 1977; Sato et al., 1977; Muller et al., 1974; Nomiyama and Nomiyama, 1971; Monster et al., 1979).

Sato et al. (1977) developed a physiological kinetic model for TCI excretion in man, based on experimental findings from controlled exposures of volunteers. Four male medical students (avg. b.w.: 61.6 kg) inhaled 100 ppm (538 mg/m^3) for 4 hours. After cessation of exposure, the blood and exhaled air concentrations of TCI and the concentration of urinary metabolites were determined during the time-course of 10-hr postexposure. Figure 4-4 shows the results obtained. The time-course of concentration decay (C) TCI in blood and exhaled air is the sum of three exponentials:

$$C_{air} = 0.213E^{-23.6t} + 0.029E^{-1.48t} + 0.0126E^{-0.18t}$$

$$C_{blood} = 0.115E^{-16.7t} + 0.449E^{-1.71t} + 0.255E^{-0.20t}$$

These reflect first-order excretion from three compartments (VRG, MG, and FG, respectively) of the model (Figure 4-4). As indicated in the model, C_{air} represents pulmonary clearance only, but C_{blood} represents whole-body clearance by the pulmonary system and metabolism. The kinetic parameters were determined as follows: V (volume of FRG, MG, and FG compartments), 8, 31, and 10/ℓ, respectively; metabolic clearance, 104 ℓ/hr; partition coefficients (blood/air, VRG/ blood, MG/blood, FG/blood), 10, 1.5, 1.0, 67; $t_{½}$ of FG, 3.4 hours. Similar tri-exponential curves for decay of blood and exhaled air concentrations of TCI have been reported by other investigators (Stewart et al., 1970, 1974; Nomiyama and Nomiyama, 1974; Fernandez et al., 1975, 1977; Monster, 1976).

The long half-time of elimination from adipose tissue (3.5 to 5 hours) explains why, after a single short exposure (e.g., 4 to 6 hours), exhaled air contains a notable concentration of TCI 18 hours later, and also why body burden of TCI occurs with repeated, fluctuating daily exposures until steady-state is reached (Section 4.1.4). Several investigators (Fernandez et al., 1977; Monster et al., 1979) have shown that, for volunteers exposed to 70 to 100 ppm (377 to 538 mg/m^3) 4 hours daily for 5 days, the concentration of TCI in blood and exhaled air was two to three times higher 18 hours after the last exposure than at the comparable time after a single exposure. TCI accumulation was highest in those volunteers with a proportionally larger ratio of adipose to lean muscle mass.

4.2.2 Urinary Metabolite Excretion in Man

The urinary metabolites of TCI (total amount, excretion ratios, and excretion time-course) have always been of considerable interest over the years as quantitative indices of exposure and body burden. Recent investigations have determined that the half-time for renal elimination of TCE and of TCE-glucuronide approximates 10 hours (range 7 to 14 hours) after TCI exposure (Nomiyama and Nomiyama, 1971; Muller et al., 1974; Sato et al., 1977; Monster et al., 1976, 1979). This value is in agreement with that observed after ingestion of TCE itself (Breimer et al., 1974; Muller et al., 1974). As might be predicted from this relatively short half-time of renal elimination, blood concentrations of TCE and TCE-glucuronide after repeated daily TCI exposures reach a "plateau concentration" (with peaks for each daily exposure) that is dependent on the inhaled air concentration of TCI (Monster et al., 1979; Muller et al., 1974; Fernandez et al., 1977). Consequently, the same daily amount of TCE and TCE-glucuronide is excreted into the urine during repeated daily exposures to the same air concentration of TCI.

In contrast to the short half-time of TCE elimination, the half-time of renal elimination of TCA produced during TCI exposure approximates 52 hours (range 35 to 70 hours) (Soucek and Vlachova, 1960; Nomiyama and Nomiyama, 1971; Muller et al., 1974; Sato et al., 1977; Monster et al., 1976). Similar values have been observed when TCA is directly administered to men (82 hours, Paykoc and Powell, 1945; 51 hours, Muller et al., 1974). Distribution of TCA in the body is restricted to the extracellular water space (Paykoc and Powell, 1945); it is very tightly and extensively bound to plasma protein and excluded from erythrocytes, so that plasma concentration is twice as high as whole blood (Soucek and Vlachova, 1960; Monster et al., 1979). As a consequence of the long half-time of TCA, and of the constant metabolic formation of TCA from TCE, blood concentrations of TCA smoothly rise during a single TCI exposure, reaching a maximum 24 to 48 hours postexposure before declining exponentially for 10 to 15 days. The blood concentration of TCA during the rising phase and at maximum is proportional to the TCI inspired air concentration and the duration of the exposure, i.e., to the retained TCI dose (Soucek and Vlachova, 1960; Monster et al., 1976; Sato et al., 1977; Fernandez et al., 1977). During repeated daily exposures, TCA accumulates in the blood exponentially, and declines only 24 to 48 hours after the last exposure (Muller et al., 1974; Fernandez et al., 1977; Monster et al., 1979). Consequently the daily amount of TCA excreted into the urine increases during repeated exposure and then decreases slowly over a period of 15 to 20 days. Several investigators have documented an apparent increase in the half-time of renal elimination of TCA (to 100+ hours) with repeated daily exposures to TCI. This phenomenon is explained by continuous formation of TCA from accumulated tissue stores of TCI and TCE, and by the very tight binding of TCA to plasma protein.

The renal elimination kinetics of TCE and TCA readily explain the urinary excretion ratio time-course of these metabolites. The ratio of daily urinary metabolites, TCE/TCA (mg/day), is highest (2:1) 24 hours after a single TCI exposure or after repeated daily exposures (Soucek and Vlachova, 1960; Muller et al., 1972, 1974; Fernandez et al., 1975; Nomiyama and Nomiyama, 1971, 1977; Sato et al., 1977; Monster et al., 1976, 1979). Thereafter, the relationship of TCE/TCA progressively decreases from day to day and becomes less than unity at about the sixth day following exposure. Several additional factors are known to influence the excretion ratio, TCE/TCA. The ratio is reported to be markedly greater in males than females for the first 24 hours after exposure (Nomiyama and Nomiyama, 1971, 1977), and TCA urinary excretion is reported to vary diurnally (Soucek and Vlachova, 1960; Monster et al., 1979). There is no evidence in man that the elimination of TCI by metabolism is saturable, at least up to 300 ppm (1584 mg/m^3) inhalation concentrations, but saturation may occur at higher levels (Section 4.4.2).

4.2.3 Excretion Kinetics in the Rodent

Withey and Collins (1980) determined the kinetics of distribution and elimination of TCI from blood of Wistar rats after intravenous (i.v.) administration of 3, 6, 9, 12, or 15 mg/kg of TCI given in 1 ml water interjugularly. For the three lower doses, the blood decay curves exhibited two components of exponential disappearance and best fitted a first-order two-compartment model, with average kinetic parameters of K_e, 0.18 min^{-1}; V_d, 158 ml; k_{12} and k_{21}, 0.059 and 0.39, respectively. For the highest dose (15 mg/kg), these investigators found that their data best fitted a first-order three-compartment model. They suggest that the shift from two- to three-compartment kinetics may have occurred either because of a dose-related alteration in the kinetic mechanisms of uptake, distribution, metabolism, and elimination, or that three-compartment kinetics were followed at all dose levels, but with lower doses, the third exponential component, representing the third compartment, was obscured by the limit of analytical sensitivity. The kinetic values for the three-compartment model (using a dose of 15 mg/kg) were k_e, 0.174 min^{-1}; V_d, 111 ml; k_{12}, k_{21}, k_{13}: 0.183, 0.164, and 0.051, respectively. The half-time, $t_{\frac{1}{2}}$, for disappearance of TCI from perirenal fat was 217 minutes. Withey and Collins (1980) state that they found no evidence in their kinetic analysis of the disposition of i.v. bolus injections of TCI into the rat of nonlinear or dose-dependent Michaelis-Menten kinetics, although the first-order kinetic parameters obtained were clearly dose-dependent. These workers suggested that a dose of 15 mg/kg TCI to the rat is below hepatic metabolism saturation. However, other investigators have found clear evidence of dose-dependent Michaelis-Menton metabolism kinetics in rats at higher doses (Section 4.4.2.2).

Prout et al. (1984) also have made observations on the overall rate of elimination of TCI from the blood of rats and of mice. After single intragastric doses of 1000 mg/kg in corn oil vehicle to rats and to mice, the blood levels of TCI and principal metabolites were monitored by GC analysis. Figure 4-5 shows the results of these experiments. Peak blood TCI occurred at 1 hour in the mouse, but rats exhibited a minor peak at 1.5 hours and a major peak at 3 hours. This absorption behavior in the rat has been previously reported by Withey et al. (1983) and is characteristic of corn oil vehicle but not of water solutions of TCI (Section 4.1.2). Following absorption and distribution (post blood peak), blood TCI disappeared (pulmonary elimination plus metabolism) in an exponential first-order manner with a half-life of about 1.75 hours for the mouse and 2.25 hours for the rat, i.e., with nearly the same rate constant in the two species. The primary hepatic metabolite, chloral (see Figure 4-5), appears in mouse blood rapidly with a major peak at 2 hours, but in the rat the peak was delayed to 10 hours; furthermore, the blood levels of chloral were 2 to 4-fold higher in the mouse than rat. These investigators suggest that these results indicate the metabolism of TCI in the mouse in considerably more rapid than that of the rat. A further difference between the mouse and rat was an apparent difference in the conversion of chloral to TCE and TCA. Prout et al. (1984) stress that the more rapid conversion of chloral to TCA in the mouse leads to an overall sustained elevation of blood levels of TCA in the mouse (~7-fold; Figure 4-5, Panel D) than in the rat, although the relative proportion of TCA to other metabolites (7 to 15 percent TCA) in urine, bile, and feces was not greater than that of the rat (Table 4-9); indeed, the metabolite proportion appears independent of dose, species, or animal strain (Green

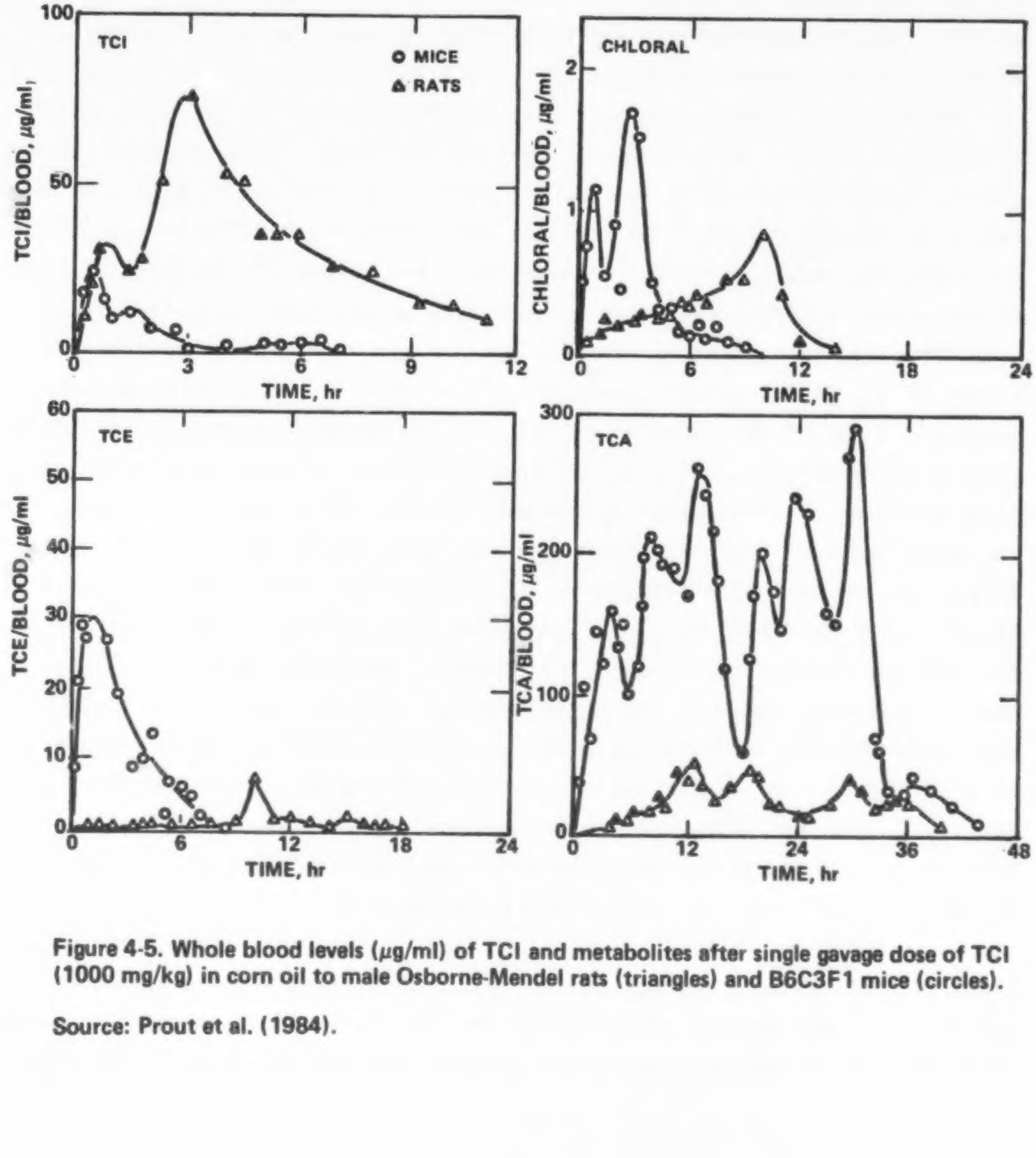


Figure 4-5. Whole blood levels (µg/ml) of TCI and metabolites after single gavage dose of TCI (1000 mg/kg) in corn oil to male Osborne-Mendel rats (triangles) and B6C3F1 mice (circles).

Source: Prout et al. (1984).

TABLE 4-9. PROPORTION OF TCI METABOLITES (TCA AND TCE) IN URINE OF RATS AND MICE AFTER SINGLE ORAL DOSES OF ^{14}C-TCI

Dose mg/kg	% of urine radioactivity[a]			
	Rat		Mouse	
	TCA	TCE	TCA	TCE
10	5.8	91.5	12.2	84.5
500	6.3	90.1	7.2	91.1
1000	8.3	88.4	7.0	88.5
2000	7.0	91.9	7.2	90.1

[a] Urine collected for 24 hours from Osborne-Mendel rats and B6C3F1 mice. Similar results from Alderly Park (Wistar derived) rats and Swiss-Webster mice.

Source: Green and Prout (1984).

and Prout, 1984). However, as seen in Figure 4-5, Panel D, the area under the TCA blood curve (AUC), as a measure of TCA production in the mouse, is 10-fold greater than the AUC for the rat, with the implication of a considerably greater production of TCA per unit dose in the mouse than rat.

Prout et al. (1984) also evaluated the excretion of TCA and of CO_2 (as measures of TCI), as a function of dose in both the rat and the mouse, respectively. Animals were dosed with a solution of ^{14}C-TCI in corn oil given as a single intragastric administration at levels of 10, 500, 1000, and 2000 mg/kg. ^{14}C-radioactivity excreted in urine as ^{14}C-TCA, and in expired air as $^{14}CO_2$ (from TCA metabolism as one source) from 0 to 24 hours, was measured. Figure 4-6 shows the results. The sum of ^{14}C-TCA and $^{14}CO_2$ radioactivity excreted by mice (B6C3F1) over the dose range 10 to 2000 mg/kg was linear with dose. In contrast, the excretion of these metabolites by rats (Osborne-Mendel) was nonlinear and rate-limited, indicating in the rat (but not mouse) dose-dependent kinetics for TCI metabolism to TCA.

Green and Prout (1984) have investigated the kinetics of urinary excretion of TCI metabolites in mice (B6C3F1) during chronic administration of TCI. Single intragastric doses of TCI in corn oil (1000 mg/kg) were administered daily for 180 days. On days 10 and 180, ^{14}C-TCI was incorporated into the dose. The 24-hr excretion of unchanged ^{14}C-TCI and of $^{14}CO_2$ in the exhaled

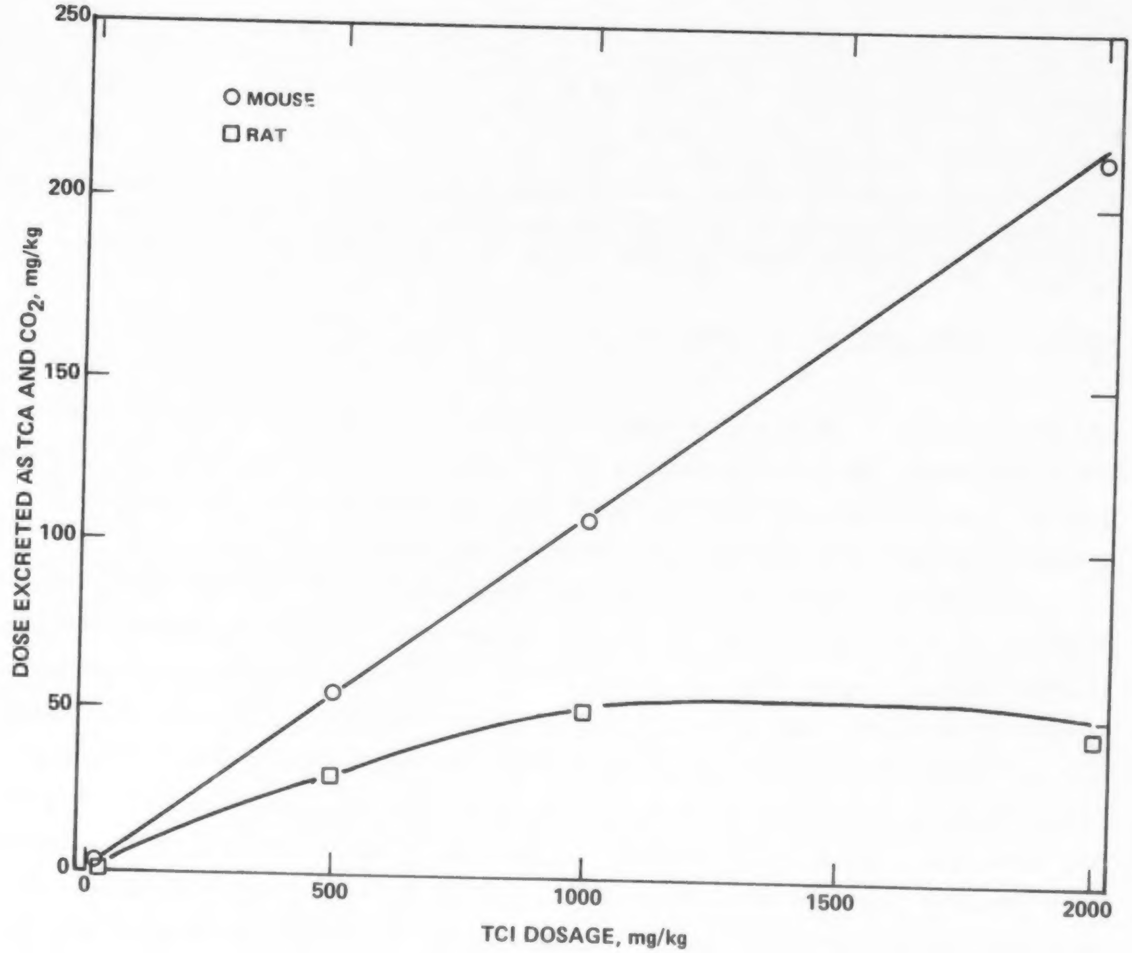


Figure 4-6. Dose-TCI metabolite excretion relationship in male B6C3F1 mice and Osborne-Mendel rats given single intragastric doses of ^{14}C-TCI in corn oil. The sum of radioactivity excreted in urine as TCA and in expired air as CO_2 within 24 hr after dosing is plotted against dosage. Each data point represents 4 animals.

Source: Prout et al. (1984).

breath, and of total ^{14}C-radioactivity in urine expressed as ^{14}C-TCA and ^{14}C-TCE, were measured. Control animals (untreated) were given single doses of ^{14}C-TCI on days 10 and 180. The results are shown in Table 4-10. Chronic dosing of TCI in mice for up to six months had no overall effect on the extent of metabolism; a similar proportion of the TCI dose was excreted through the lungs unchanged, and a similar proportion was metabolized ($^{14}CO_2$ in exhaled air plus total radioactivity in urine) after six months to that metabolized after a single dose. These observations indicate that at this relatively high dose (1000 mg/kg), induction of TCI metabolism in response to chronic administration did not occur, although TCI was undergoing nearly maximal (80 percent) metabolism in any case. Hence, these observations might not pertain to "saturating" doses of TCI (>2000 mg/kg; Figure 4-7). However, although there was no overall increase in the proportion of TCI metabolized, a change in the relative amounts of the major metabolites (TCA and TCE) in urine was observed. After 10 days of dosing and thereafter, the amount of TCA excreted in the urine was double that found after a single dose (Table 4-5), while TCE (as the glucuronide) decreased in proportion. These observations suggest that the oxidative metabolism of chloral to TCA is increased at the expense of the reductive pathway of TCE with chronic dosing as suggested by Green and Prout, or alternatively, the kinetics of urinary excretion of TCA and TCE do not achieve a steady-state with daily dosing of TCI until after 10 days, because of a relatively long half-time for renal excretion of TCA. A similar phenomenon has been observed in the human (Section 4.2.2) and in the rat (Muller et al., 1972, 1974).

4.3 MEASURES OF EXPOSURE AND BODY BURDEN

Trichloroethylene is principally absorbed by the lungs during industrial or ambient air exposure. However, as worker exposure periods and air concentrations are likely to vary, air analysis does not necessarily provide a reliable indication of true exposure. An index of a worker's current exposure and total body burden can be estimated in accordance with the amount of TCI absorbed and retained as predicted by the known pharmacokinetics and metabolism of TCI. Therefore, monitoring TCI levels in blood or expired air and measuring urinary metabolites are the most common methods for determining the body burden (Stewart et al., 1962, 1970, 1974; Morgan et al., 1970; Nomiyama, 1971; Ogata et al., 1971; Ertle et al., 1972; Ikeda et al., 1972; Muller et al., 1972, 1974;

TABLE 4-10. EFFECT OF CHRONIC DAILY ORAL DOSING OF TCI (1000 mg/kg) ON METABOLISM AND METABOLITE EXCRETION IN B6C3Fa MICE*

Day	Unchanged TCI	CO_2	Urine	Feces	Urine TCA	Urine TCE
	As % of Dose (24-hr Collection)				As % of ^{14}C-TCI Radioactivity in 24-hr urine collection	
1	17.5 ± 8	6.0 ± 0.7	41.8 ± 6.5	13.1 ± 9.5	7.0	88.5
10	13.6 ± 8.6	6.3 ± 0.6	52.3 ± 7.8	16.4 ± 3.3	15.6	81.9
Control**	15.4 ± 8.6	5.3 ± 1.0	46.9 ± 10	12.3 ± 1.9	8.4	88.1
180	23.7 ± 6.2	2.3 ± 1.0	48.3 ± 6	1.9 ± 0.5	19.9 ± 3.1	77.4 ± 2.8
Control	12.5 ± 2.0	4.2 ± 0.6	50.7 ± 5.1	1.3 ± 0.5	10.5 ± 2.4	86.9 ± 1.8

*N = 4 test, N = 3 control.

**Test and untreated control mice received single doses of ^{14}C-TCI (1000 mg/kg: 10 μCi) on day 10 and day 180.

Source: Green and Prout (1984).

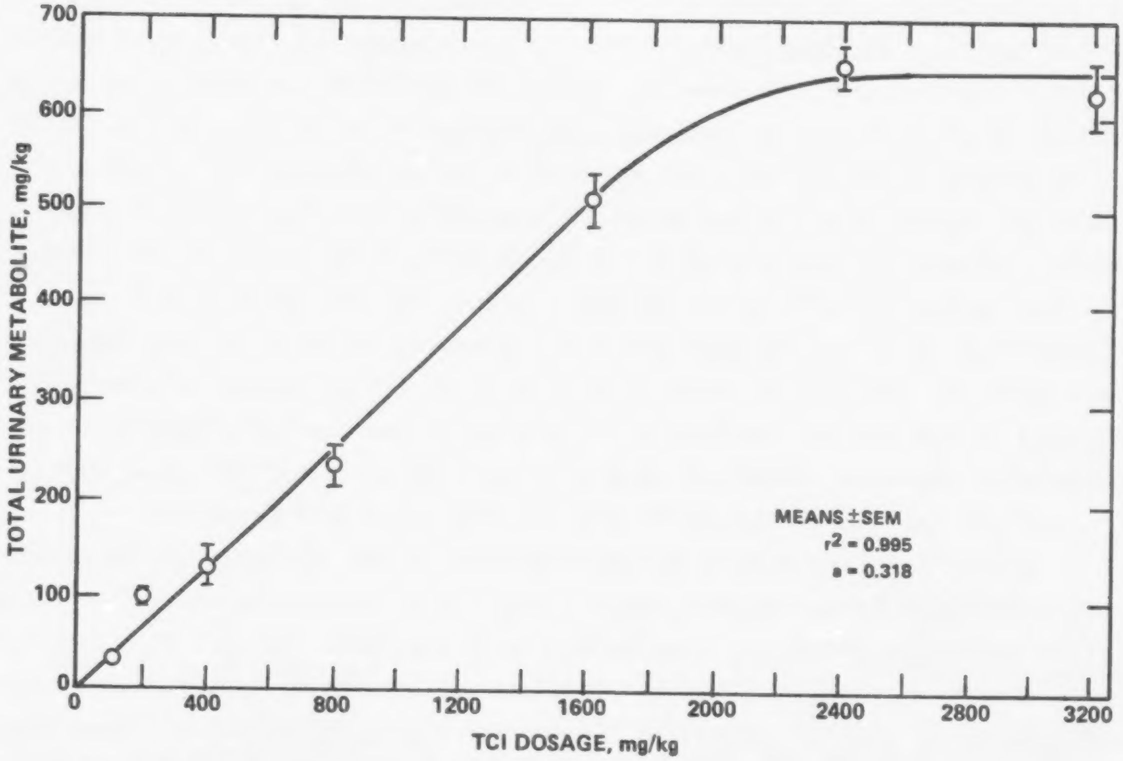


Figure 4-7. Relationship between TCI dose and the amount of total urinary metabolite excreted per day by mice in each group. Values represent means ± SEM. N = 7-9 mice per group except for the 100 and 3200 TCI/kg groups where N = 3 and 4 respectively. The slope (0.318) and r^2 value coefficient (0.996) are for linear regression fit of the 100 - 1600 mg/kg data points.

Source: Buben and O'Flahery (1984).

Pfäffli and Blackman, 1972; Kimmerle and Eben, 1973a,b; Lowry et al., 1974; Vesterberg et al., 1976). These methods, however, are subject to high interpersonal variations in the pharmacokinetics and metabolism of TCI that limit their predictive value. Some of the factors now known to contribute to these variabilities are pulmonary dysfunctional states, interindividual differences in intrinsic liver metabolism capacity for TCI, modification of metabolism by drugs and environmental xenobiotics, age, sex, exercise or work load, and body anthropometry. Stewart and his associates (1974) have been vigorous proponents of the value of postexposure alveolar concentrations of TCI as measures of recent exposure and body burden. However, this method is highly dependent on time of sampling and influenced by individual variations in metabolism of TCI. Muller et al. (1974) have concluded that neither TCA excretion nor the sum of urinary metabolites can be taken as representative parameters of previous exposure, except possibly for 24-hr daily collections. These workers favor either alveolar TCI concentrations or blood analysis of TCA or TCI as the best present indicators of exposure and body burden. Monster et al. (1979) concluded from their studies that the most "promising parameter for biological monitoring in repeated exposure to TCI" is blood TCA concentration level, because of the ease of sampling and GC analysis, the greater independence of time after exposure, accumulation with chronic exposure and increased body burden, and the smaller interindividual variation of this parameter.

Greater and more precise information than is now available on the absorption, distribution and metabolism kinetics of TCI is required for better means of estimating or predicting body burdens with exposure. To this end, many investigators have developed mathematical models formulating the pharmacokinetics of inhaled TCI and its metabolism (Fernandez et al., 1977; Droz and Fernandez, 1978; Feingold and Holaday, 1977; Gobbato and Mangivachhi, 1979; Sato et al., 1977; Sato, 1979).

4.4 METABOLISM

4.4.1 Known Metabolites

It has been known for 50 years that TCI is extensively metabolized in the body to TCE, TCE-glucuronide ("urochloralic acid"), and TCA. Following the introduction in the 1930's of TCI as a general anesthetic, Barrett and coworkers (1939) isolated TCA from the urine of dogs and human patients anesthetized with TCI. Powell (1945, 1947) demonstrated the presence of TCA in both plasma and urine. She also observed that TCA continued to be excreted for more than 2 days after a single anesthetic exposure. Butler (1949), who had previously discovered that the hypnotic, chloral hydrate, was metabolized to TCE, TCE-glucuronide, and TCA (Butler, 1948), hypothesized that this widely used hypnotic was an intermediate metabolite of TCI. His experiments with dogs exposed to TCI clearly demonstrated that the principal metabolites of TCI and TCE were TCE-glucuronide and TCA. However, Butler could not demonstrate the presence of chloral hydrate in either plasma or urine. Nonetheless, this demonstration of biotransformation of TCI to TCE as well as to TCA was the first proof that TCI anesthetic was converted to a metabolite with hypnotic properties. Trichloroethanol has a hypnotic potency comparable to that of chloral hydrate (Marshall and Owens, 1954). Since that time, the intermediate metabolite, chloral hydrate, has been found in the plasma of rats (Kimmerle and Eben, 1973a) and humans inhaling TCI (Cole et al., 1975), and has been shown to be produced in the isolated rat liver upon perfusion with TCI (Bonse et al., 1975) as well as with rat liver microsomal preparations (Byington and Liebman, 1965; Ikeda et al., 1980; Miller and Guengerich, 1982) and with isolated hepatocytes (Costa and Ivanetich, 1984).

The principal site of metabolism of TCI is the liver, although Dalbey and Bingham (1978) have demonstrated that the rat and guinea pig lung also metabolize TCI to TCE, and TCA. Other organ tissues, such as kidney, spleen and small intestine, may also metabolize TCI, as these have been shown to be sites of cellular protein binding of metabolites from TCI (Bolt and Filser, 1977; Banerjee and Van Duuren, 1978; Stott et al., 1982).

In addition to the major end-product metabolites of TCI, chloral, TCE, TCE-glucuronide and TCA, a number of other metabolites have been found in urine or exhaled breath (CO, CO_2, monochloro- and dichloroacetic acids, TCA-glucuronide, oxalic acid, N-(hydroxyacetyl) aminoethanol (HAEE), chloroform) or in vitro with microsomal systems (TCI-epoxide, glyoxylic acid, CO). These metabolites are listed in Table 4-11. Except for HAEE, oxalic acid, TCA-CoA conjugate, and TCA-glucuronide, these metabolites have been established by more than one investigator. However, chloroform, which may be an artifact of analytical methodology, needs further confirmation.

TCI-epoxide (1,1,2-trichloroethene oxide) is known to decompose in aqueous physiological conditions (Table 4-11) to formic acid, dichloroacetic acid, glyoxylic acid, and CO. These metabolites may, therefore, be formed at cellular extra-microsomal sites of TCI-epoxide formation under aqueous conditions.

TABLE 4-11. IDENTIFICATION BY GC/MS OF TCI METABOLITES IN EXHALED AIR AND URINE OF WISTAR RATS AND NMRI MICE AFTER A SINGLE ORAL DOSE OF 200 mg/kg OF ^{14}C-TCI

	Rat % of dose radioactivity	Mouse % of dose radioactivity
Exhaled air		
Unchanged ^{14}C-TCI	52.0	11.0
$^{14}CO_2$	1.9	6.0
Urine	41.2	76.2
	% of urine radioactivity	
Oxalic acid[a]	1.3	0.7
Dichloroacetic acid	2.0	0.1
N-(Hydroxyacetyl)-aminoethanol (HAAE)	7.2	4.1
Trichloroacetic acid	15.3	0.1
Trichloroethanol (free)	11.7	0.1
Trichloroethanol (conjugated)	61.9	94.2

[a] Metabolites identified by GC-MS; female Wistar rats and NMRI mice; collec-- tions for 72 hr.

Source: DeKant et al. (1984).

Until recently, a thorough chemical isolation and identification of TCI metabolites by rigorous methodologies (GS-MS, etc.), in one or more species, had not been attempted. Dekant et al. (1984) have now reported their results of a comparative study in rats and mice of ^{14}C-TCI metabolites in breath and in urine, where metabolite isolation was accomplished by reverse-phase HPLC and identification by radio-GC and GC-MS. The metabolites identified and their relative abundance are given in Table 4-11. All metabolites found in the rat were also found in the mouse. The urine radioactivity represented 41 and 76 percent of the total radioactivity given to rats and mice, respectively, from a 200-mg ^{14}C-TCI/kg dose. The radioactivity in feces represented less than 5 percent of the dose with total recovery in urine, feces, and breath of 93 to 98 percent. Two new urinary metabolites were found in both species, oxalic acid and HAAE. Dekant et al. also identified significant amounts of HAAE in the urine of human volunteers exposed to 200 ppm (1096 mg/kg) TCI for 6 hours. However, the occurrence of oxalic acid in urine and of CO_2 in breath have not as yet been identified in man as specific metabolites of TCI; to do so would require administration of labeled TCI (radioactive ^{14}C-TCI or stable isotope ^{14}C-TCI) since both compounds are also normal endogenous compounds of intermediary metabolism.

The data of Dekant et al. (Table 4-11) would indicate that TCA is not a prominent urinary metabolite of TCI in NMRI mice, in comparison to Wistar rats (or man, Section 4.2.2). Green and associates (1982, 1984) have postulated a TCA-CoA conjugate in the mouse, which is slowly excreted via the biliary system with the feces. Dekant et al. (1984) have also suggested that an intense enterohepatic recirculation of TCA (as an alkali-labile conjugate) may occur in this species. However, the small percentage of dose radioactivity appearing in the feces of the mice (<5 percent), would not indicate this route is important in the excretion of TCA.

In contrast to the observations of Dekant et al., Green and Prout (1984) found a much greater relative proportion of TCA to TCE in mice (Table 4-9), and also they found no difference in the proportion (TCA/TCE) between mouse and rat species, or between different strains of these species. The proportions were unaffected after single oral doses between 10 and 2000 mg/kg. Furthermore, the proportion of TCA/TCE increased with chronic exposure to TCI in the mouse (Green and Prout, 1984), an observation which has been shown also for the rat and for man (Section 4.2.2). Green and Prout (1984) found that not only were the relative proportions of the major metabolites TCA and TCE in urine very similar in rats and mice but also minor metabolites (dichloroacetic acid, monochloroacetic acid, free TCE) were also similar, and they therefore concluded that there is not a major difference in pathways of TCI metabolism in these two species.

4.4.2 Magnitude of TCI Metabolism: Evidence of Dose-Dependent Metabolism

4.4.2.1 Man. Estimates of the extent of metabolism in man, as a percentage of retained TCI, have been made from balance studies, i.e., accounting for a retained dose after vapor exposure by measuring pulmonary elimination of TCI and metabolites excreted into the urine. Balance studies with isotopically labeled TCI have not been reported in man. Man appears to metabolize inhaled TCI extensively (between 40 to 75 percent of the retained dose) as reported by various investigators over the last three decades (Barrett et al., 1939; Powell, 1945; Forssman and Holmqvist, 1953; Soucek and Vlachova, 1960; Bartonicek, 1963; Ogata et al., 1971; Ertle et al., 1972; Muller et al., 1972, 1974, 1975; Kimmerle and Eben, 1973a,b; Stewart et al., 1974; Fernandez et al., 1975, 1977; Vesterberg et al., 1976; Nomiyama and Nomiyama, 1971, 1974a,b, 1977; Sato et al., 1977; Monster et al., 1976, 1979). These studies do not present any evidence to indicate that a saturation of metabolism occurs in man. The data of Nomiyama and Nomiyama (1977) (Figure 4-8) and of Ikeda (1977), which correlate urinary metabolite excretion with increasing levels of TCI exposure, indicate that liver capacity for metabolism of TCI is nonlimiting, at least up to an exposure of 315 ppm (1695 mg/m^3) for 3 hours (equivalent to 25 mg TCI/kg b.w.). Furthermore, the alveolar to inspired air concentration ratio (25 percent; retention 75 percent) at exposure equilibrium approximates the cardiac output flow through the liver (25 percent) and suggests that TCI is completely removed from blood by the liver in a single pass. However, Feingold and Holaday (1977) predicted from mathematical simulation models of TCI at anesthetic concentration in man (2000 ppm; arterial concentration, 9.9 mg/dl) that metabolism was rate-limited by saturation to 49 percent of uptake for an 8-hr exposure.

The problems encountered with balance studies in man are the difficulties associated with accurate measurement of the retained dose of TCI from vapor exposure, and the imprecision of the older methodologies for determinations of urinary metabolites by extraction and colorimetry using modifications of the Fujiwara reaction. More recent careful studies, using GC methods, show that after single or repeated daily exposures from 50 to 380 ppm (274 to 2082 mg/m^3), an average of 11 percent of retained TCI is eliminated unchanged by the lungs ($t_{\frac{1}{2}}$, 5 hr), 2 percent of the dose is eliminated as TCE by the lungs ($t_{\frac{1}{2}}$, 10 to 12 hr), and 58 percent is eliminated as urinary metabolites (Fernandez et al., 1975, 1977; Muller et al., 1977; Monster et al., 1976, 1979). Unaccounted for is nearly 30 percent of the TCI dose. These investigators suggest additional pathways or routes of elimination of one or more unknown metabolites. This suggestion is particularly apropos in light of the recent demonstration

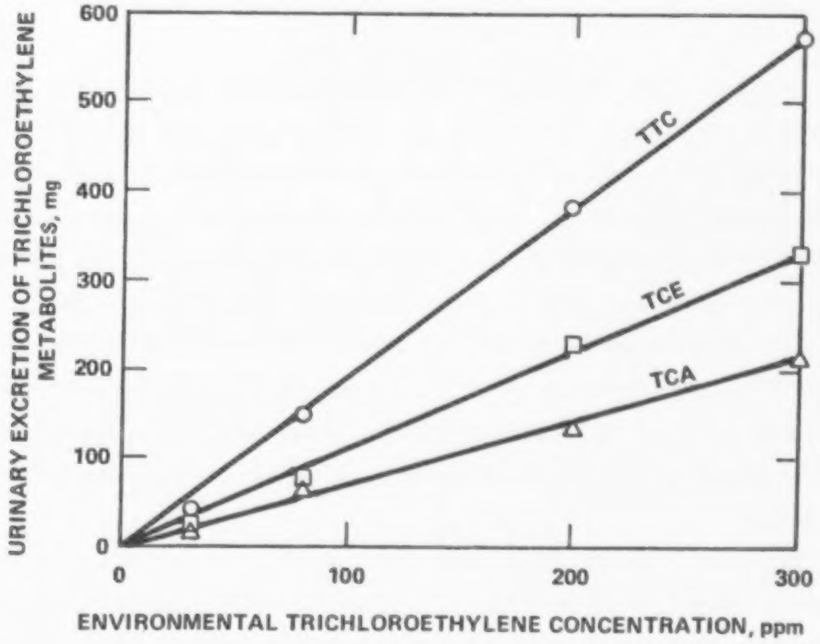


Figure 4-8. Relationship between environmental TCI concentration and urinary excretion of TCI metabolites in human urine. Urine was collected for 6 days after exposure. (Adapted from Nomiyama and Nomiyama, 1977).

by Dekant et al. (1984) of two new metabolites of TCI excreted in the urine of rats and mice, oxalic acid and HAEE. Other reported but unconfirmed minor urinary metabolites of TCI, such as chloroform (1 percent in rats, Muller et al., 1974) and monochloroacetic acid (4 percent in man, Soucek and Vlachova, 1960) do not account for the discrepancy. On the other hand, early studies in dogs by Owens and Marshall (1955) show that TCE-glucuronide is concentrated and secreted in bile. Several studies also show that TCE or TCA given orally or intravenously is not fully recovered in the urine (Marshall and Owens, 1954; Owens and Marshall, 1955; Paykoc and Powell, 1945; Muller et al., 1974). Bartonicek (1962) found that 8.4 percent of a TCI dose in humans is excreted in the feces as TCE and TCA. Part of the unaccounted TCI dose may also be explained by gastrointestinal excretion; TCI can be expected to be rapidly distributed into the GI lumen because of its high lipid solubility and ubiquitous distribution. In any case, these studies indicate at least 60 percent and possibly as much as 90 percent of retained TCI from vapor exposures of less than 500 ppm (2690 mg/m^3) is metabolized in the body.

4.4.2.2 Animals. There is an increasing body of experimental evidence that the magnitude of metabolism of TCI by rodents (rat and mouse) is dose-dependent and possibly best described by Michaelis-Menten metabolism kinetics. Thus, at very high doses, TCI metabolism exhibits saturation kinetics in both the mouse and the rat, but differently, in that evidence of approaching saturation occurs in mice at about 2000 mg/kg (absolute dose--60 mg/animal), and in rats at about 500 to 1000 mg/kg (absolute dose--125-250 mg/animal). Hence, both species have a considerable capacity to metabolize TCI. Furthermore, the maximal capacities of the rat versus the mouse appear to be more closely related to relative body surface areas (on an absolute dose/animal basis) than to body weight.

The types of studies that have been done to investigate dose-metabolism relationships are fourfold: 1) single-dose studies across species using ^{14}C-TCI and urinary radioactivity and/or other methods as measures of metabolism, 2) kinetic models based on inhalation kinetics, 3) comparative balance studies between rat and mouse with ^{14}C-TCI at 1 or 2 dose levels, 4) multiple dose levels studies in rat and/or mouse.

Across species: Early studies by Butler (1948) demonstrated the extensive metabolism of TCI in dogs for which he estimated the extent of metabolism (as a percentage of the administered dose) to be 60 to 90 percent. Muller et al. (1982) recently compared the metabolism of TCI in chimpanzees, baboons, and rhesus monkeys. ^{14}C-TCI was administered by intramuscular injection at a dose level of 50 mg/kg. Radioactivity excreted in urine and feces (as an index of metabolism) ranged from 40 to 60 percent of the dose in chimpanzees, 11 to 28 percent in baboons, and 7 to 40 percent in rhesus monkeys. Their results showed a significant difference in excretion route between man/chimpanzee and rhesus/baboon. While man and chimpanzee excreted only from 2 to 5 percent of the total eliminated TCI metabolites in the feces, baboon and rhesus monkey excreted 18 to 35 percent of the radioactivity in the feces. Rodents have been reported to excrete in feces 3 to 8 and 17 to 19 percent for the rat and mouse, respectively, of a radioactive dose of TCI (Table 4-12, Prout et al., 1984). Green and Prout (1984) have directly demonstrated by bile duct cannulation that TCI metabolite(s) are excreted in bile by both rats and mice.

TABLE 4-12. DEMONSTRATED METABOLITES OF TCI (OTHER THAN CHLORAL AND CHLORAL DERIVATIVES: TCE, TCE-GLUCURONIDE, AND TCA)

Metabolite	System	Investigator
TCI-epoxide	rat, mouse microsomes	Miller and Guengerich, 1982
Glyoxylic acid	rat, mouse microsomes	Miller and Guengerich, 1982
Carbon monoxide	rat, mouse microsomes	Miller and Guengerich, 1982 Traylor et al., 1977 Fetz et al., 1978
Carbon dioxide	exhaled air rat and mouse	Parchman and Magee, 1982 Stott et al., 1982 Dekant et al., 1984 Green and Prout, 1984
Monochloroacetic acid	human and rabbit urine	Soucek and Vlachova, 1960 Ogata and Saeki, 1974
	mouse and rat urine	Green and Prout, 1984
Dichloroacetic acid	rat and mouse urine	Hathaway, 1980 Dekant et al., 1984 Green and Prout, 1984
Oxalic acid	rat and mouse urine	Dekant et al., 1984
N-(Hydroxyacetyl)-aminoethanol (HAAE)	rat, mouse, human urine	Dekant et al., 1984
TCA-glucuronide	chimpanzee urine	Muller et al., 1982
TCA-CoA	mouse feces, bile	Green et al., 1982 Green and Prout, 1984
Chloroform	rat adipose tissue, serum, urine	Pfaffenberger et al., 1980 Muller et al., 1974
Products from TCI-epoxide decomposition in aqueous solution, at 37°C		
Formic acid Dichloroacetic acid Carbon monoxide Glyoxylic acid		Henschler et al., 1977, 1978 Kline and Van Duuren, 1977 Bonse et al., 1975 Miller and Guengerich, 1982

Inhalation kinetics: Filser and Bolt (1979) have exposed rats (Wistar) to TCI in a closed system (desiccator jar chamber) and determined, from disappearance of TCI from the chamber the inhalation pharmacokinetics, in accordance with the following model:

$$\begin{tikzcd}
V_1 \arrow[r, shift left, "k_{12}"] & V_2 \arrow[l, shift left, "k_{21}"] \arrow[r, "K_m", "V_m"'] & \text{metabolism.} \\
\text{Chamber atmosphere} & \text{rat} &
\end{tikzcd}$$

They found that the metabolism of TCI was dose-dependent in the rat, with the saturation point occurring at 65 ppm (350 mg/m^3). Zero order V_{max} was 210 µmol/hr/kg and first-order clearance (below 65 ppm) was 77 L/hr/kg b.w.

Andersen et al. (1980) also analyzed the pharmacokinetic behavior of TCI in rats (Fischer 344) using a Michaelis-Menten approach to demonstrate dose-dependent metabolism. Exposures were conducted in a 31-L battery jar chamber, and the rate of depletion of atmosphere TCI was determined for 30, 100, 1000, 3500, and 8000 ppm (116, 538, 5380, 18,830 and 43,040 mg/m^3). TCI uptake was fitted to a four-compartment model as follows:

$$\begin{tikzcd}
\text{TCI atmosphere} \arrow[r, shift left, "k_{12}"] & \text{Blood, liver, etc.} \arrow[l, shift left, "k_{21}"] \arrow[r, shift left, "k_{23}"] & \text{Fat, bone, etc.} \arrow[l, shift left, "k_{32}"] \\
 & K_m & \\
 & \text{Metabolites} &
\end{tikzcd}$$

The metabolism of TCI was found to be dose-dependent, with a saturation point of about 1000 ppm (5380 mg/m^3) (K_m, 463 ppm). The kinetic parameters, calculated as molar equivalents TCI, were V_{max}, 185 µmoles/kg/hr; K_m, 292 µmoles/hr/kg for uptake from 31-L chamber. A "whole animal"/air partition coefficient of 15.4 (as compared to 9.0 for blood/air) was calculated and suggested to be a more accurate estimate of microsomal-to-gas distribution than a blood/gas constant.

Balance studies: For rodents (rat and mouse), the extent of metabolism after single doses of TCI has been estimated by balance studies using isotopically labeled TCI. Daniel (1963) carried out the earliest study in rats. The rats were dosed by stomach tube with $^{36}C\ell$-labeled TCI liquid (40 to 60 mg/kg b.w.); 80 percent of the dose was eliminated via the lungs with a half-time of elimination of about 5 hours; urinary metabolites TCE, TCE-glucuronide, and TCA accounted for only 15 percent of the dose. More recent studies show considerably different results.

Dekant et al. (1984) have compared the metabolism of TCI by balance studies, with female rats (Wistar) and with female mice (NMRI). These results are shown in Table 4-9. ^{14}C-TCI was administered as a single dose by gavage in corn oil at 200 mg/kg. The absolute dose given to the rats, based on their experimental weights, was about 48 mg and to the mice about 5.1 mg. Radioactivity was determined in urine, feces, carcass, and exhaled air for 72 hours post administration. Virtually complete oral absorption occurred with a total recovery of radioactivity of 93 to 98 percent. For this single dose, rats excreted through the lungs 52 percent of the dose unchanged (~25 mg), while the mice excreted only 11 percent (~0.56 mg). Hence, the rats metabolized an average of 48 percent of their dose (23 mg) and the mice 89 percent (4.54 mg). The ratio of the amounts metabolized by the rat versus the mouse (5.05) closely approximates their relative surface areas, which as calculated from their experimental body weights $(\text{rat b.w./mouse b.w.})^{2/3}$ is 4.46. Therefore, the metabolic capacity of rats and mice for TCI appears to be proportional to their surface areas. On a mg/kg basis the mice would appear to metabolize TCI to an extent 1.9 times greater than rats. Furthermore, the high proportion of the dose (52 percent) excreted unchanged via the lungs would suggest that a single, orally administered 200 mg/kg dose exceeded the overall hepatic capacity of the rat resulting in a significant first-pass effect.

Stott et al. (1982) exposed rats and mice to 10 and 600 ppm (54 and 3228 mg/m^3) ^{14}C-TCI for 6 hours. Their results are given in Tables 4-6 and 4-7. For exposed mice, virtually 100 percent of the body net uptake was metabolized with no evidence of metabolic saturation. The body burden (dose) from the 6-hr exposure was estimated as 78.5 µmol of TCI/kg body weight (10 mg/kg) and 3138 µmol of TCI/kg (412 mg/kg), respectively. In contrast, for rats 98 percent of the net uptake from 10 ppm exposure was metabolized but only 79 percent at 600 ppm (3228 mg/m^3) exposure, suggesting an incremental approach to saturation of metabolism in this species. For rats, the inhalation doses were 35.8 µmol/kg (4.7 mg/kg) and 1075 µmol/kg (141 mg/kg), respectively. These results indicate a saturation of metabolism in the rat. Support for this conclusion is found also in the observation that pulmonary elimination of unchanged TCI was only 2 percent of the dose from 10 ppm (54 mg/m^3) exposure but 21 percent of the dose at 600 ppm (3228 mg/m^3) (Table 4-7). In absolute amounts, mice metabolized 2.2 times more TCI per kg body weight than rats after exposure to 10 ppm, and 3.6 times more after exposure to 600 ppm (3228 mg/m^3) (Tables 4-6 and 4-7). However, on a relative body surface area basis, the ratio for the amounts metabolized by the rat versus the mouse approximated their relative body surface areas.

Multiple dose studies: Dose-dependent kinetics as opposed to first-order kinetics requires multiple dose level studies to rigorously differentiate first-order kinetics and to appropriately evaluate the associated kinetic parameters. Two investigative groups, Prout and Provan (1984) and Buben and O'Flaherty (1984), recently have used this approach to examine the kinetics of TCI metabolism in rats and in mice.

Buben and O'Flaherty administered doses of 0, 100, 200, 400, 800, 1600, 2400, and 3200 mg/kg of TCI in corn oil by intragastric intubation to male Swiss-Cox mice in groups of 7 to 9 mice. The control groups consisted of 24 mice. These doses were given 5 days/week for 6 weeks. The subchronic dosing ensured that steady-state conditions for metabolism of TCI and for metabolite(s) excretion were achieved. Metabolism was measured by determining 24-hr urine collections of TCA, TCE, and TCE-glucuronide, as determined by GC. Of the two other metabolites assayed, ethylene glycol proved negative, and oxalic acid was not increased over excretion by control animals. Metabolites in feces represented less than 5 percent of the amount in urine. No significant day-to-day variability in urinary metabolites excreted or week-to-week trends was found.

The use of "total urinary metabolites" (TCA, TCE, and TCE-glucuronide) as an index of metabolism is an approximation, as stressed by Buben and O'Flaherty themselves, because there is experimental evidence that HAEE and oxalic acid may be significant urinary metabolites of mice, 0.7 and 4.1 percent, respectively (Table 4-12), and CO_2 in exhaled breath may amount to 6 to 9 percent of the TCI dose (Tables 4-2, 4-6, and 4-12).

Thus, "total urinary metabolites" may underestimate actual metabolism by at least 20 percent or more. However, urinary TCA, TCE, and TCE-glucuronide excretion, as the principal metabolites, provides an appropriate reflection of overall TCI metabolism.

Figure 4-7 shows the relationship between dose and metabolism ("total urinary metabolites") found by Buben and O'Flaherty in mice. Up to a dose of about 1600 mg/kg, a linear relationship between dose and amount metabolized obtained with a constant 27.5 percent of the dose metabolized to the urinary metabolites TCA, TCE, and TCE-glucuronide. This percentage of dose metabolized is considerably lower than that observed above by Dekant et al. (1984) and Stott et al. (1982) for mice. Above the 1600 mg/kg dose, the metabolism of TCI shows a rapid decrease of the percent of dose metabolized and approached a saturation of metabolism at about 2400 mg/kg. These observations would indicate that TCI follows first-order metabolism (proportion to dose) in the mouse up to a relatively high dose of 1600 mg/kg (~48 mg/mouse) and thereafter dose-dependent and then zero-order kinetics follow at higher doses. Buben and O'Flaherty did not fit their data to the Michaelis-Menten equation, although it would have been instructive.

Prout et al. (1984) have carried out a similar investigation in rats (Osborne-Mendel and Alderly Park Wistar) and in mice (B6C3F1 and Swiss Webster). However, in this case the dosage series (10, 500, 1000, and 2000 mg/kg) was given as single doses (i.e., not subchronically) in corn oil by stomach tube as ^{14}C-TCI. The metabolism can be expected therefore to reflect that of single doses and not of steady-state metabolic conditions. But the use of ^{14}C-TCI allows a complete estimate of total metabolism. Thus, Prout et al. (1984) measured ^{14}C-radioactivity (expressed as percent of dose) in urine, feces, and expired air over a collection period of 72 hours, with total recovery of administered dose-radioactivity of 92 to 98 percent. Their results are tabulated in Table 4-2 for Osborne-Mendel rats and for B6C3F1 mice; entirely similar results were observed for Wistar rats and Swiss-Webster mice, i.e., the disposition of TCI was independent of rat or mouse strain. The data of Table 4-2 indicate that for both the rat and mouse, the percent of the dose metabolized decreased with increase of dose size, and in tandem, the percent of the dose excreted via the lungs as unchanged TCI increased with increase of dose size. However, these results were more evident and occurred to a much greater extent in the rat than in the mouse. Thus, a plot of dose against the portion of the dose metabolized, Figure 4-9, indicates that for the rat the metabolism of TCI approaches saturation at a dose of about 1000 mg/kg (~200 mg/animal), while for the mouse the metabolism of TCI is still linear up to a dose of 2000 mg/kg (~60 mg/animal). These results with the mouse are in

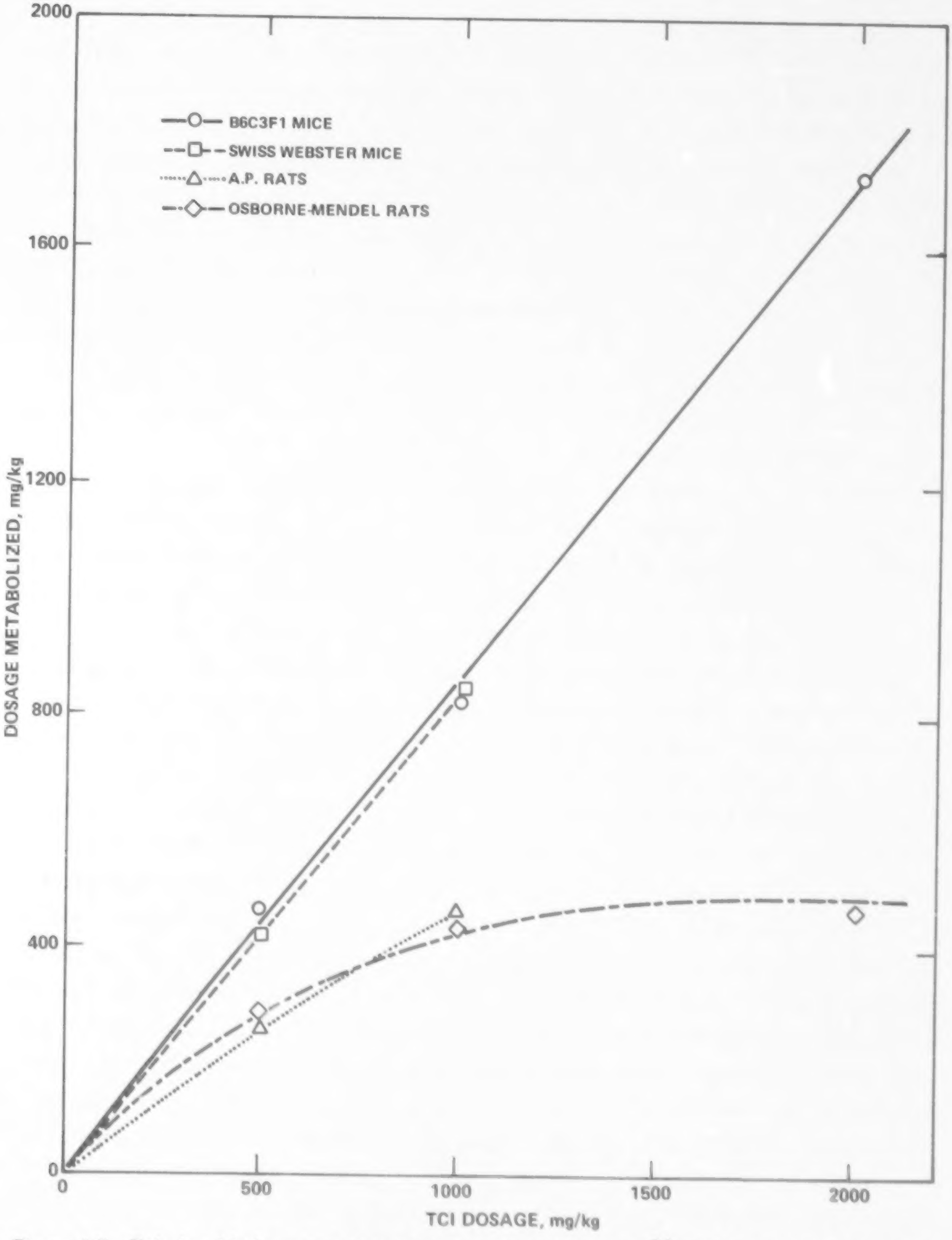


Figure 4-9. Relationship between administered single oral doses of ^{14}C-TCI to rats and mice and amount of dose metabolized in 24 hr, expressed as mg/kg b.w., as calculated from ^{14}C- radioactivity excreted in urine, feces, and expired air (other than unchanged ^{14}C-TCI). Each data point represents 4 rats or mice.

Source: Prout et al. (1984).

accord with those of Buben and O'Flaherty, who found that metabolism was not saturated in mice up to doses of 2400 mg/kg (Figure 4-7). It can be concluded, therefore, that metabolism of TCI is dose-dependent and saturable at doses above 500 to 1000 mg/kg in the rat and 1600 to 2400 mg/kg in the mouse. Both species, therefore, exhibit a very high hepatic capacity to metabolize TCI. At lower doses in these species, metabolism is nearly linearly related to TCI dose, i.e., "first-order" metabolism prevails. However, it is likely that in both species TCI metabolism can be best described overall by Michaelis-Menten kinetics, particularly at high dose levels.

4.4.3 Enzyme Pathways of Biotransformation

Current knowledge of the details of the enzymatic biotransformation of TCI derives mainly from *in vitro* studies, principally with liver cell fractions, and from *in vivo* studies with end metabolite identification in exhaled breath, urine, feces, and bile. Figures 4-10, 4-11, and 4-12 summarize the probable pathways involved in the metabolism of TCI as determined from those studies. Among the halogenated hydrocarbons, TCI biotransformation appears to be unusual, since neither dehalogenation nor removal of carbon atoms occurs in the first step. The initial biotransformation of TCI involves metabolism to chloral hydrate and to formation of trichloroethylene epoxide (Figure 4-10). The formation of chloral/epoxide is the rate-limiting step in the metabolism of TCI (Ikeda et al., 1980; Nomiyama and Nomiyama, 1979). Intramolecular rearrangement with migration of a chlorine atom is presumed to occur in the formation of chloral hydrate, in order to explain the genesis of TCE and TCA. Conversion of chloral hydrate to TCE and TCA is shown in Figure 4-11.

4.4.3.1 Formation of chloral--Byington and Leibman (1965) conclusively identified chloral hydrate as the product of TCI when incubated with rat liver microsomes fortified with NADPH and O_2 (P_{450} mono-oxygenase system), thereby verifying the hypothetical chloral hydrate formation proposed by Butler nearly 20 years earlier. These workers also postulated an intermediate formation of an epoxide (as did Powell in 1945), analogous to the known microsomal oxidation to epoxides of dihydronaphthalene, aldrin, heptachlor and isodrin. Costa and Ivanetich (1984) found that chloral hydrate was the major metabolite in isolated TCI-treated hepatocytes from phenobarbital-pretreated or untreated rats. Daniel (1963) provided evidence that chloride migration must occur in chloral formation since no dilution of ^{36}Cl atoms with chlorine atoms from other body

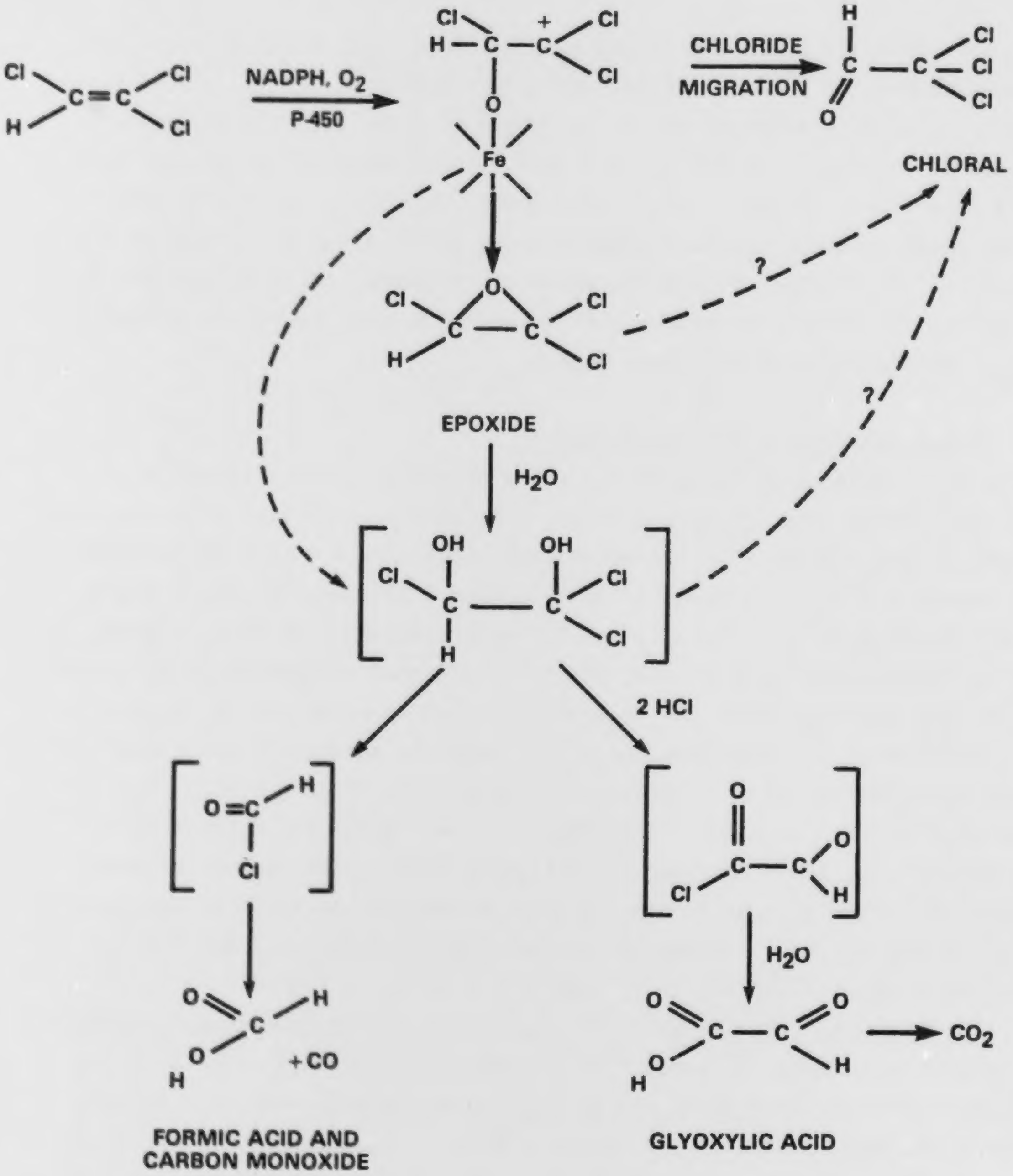


Figure 4-10. Postulated scheme for the metabolism of trichloroethylene to chloral (see Figure 4-11 for chloral metabolism) and to trichloroethylene epoxide and its metabolites.

Source: Miller and Guengerich (1982).

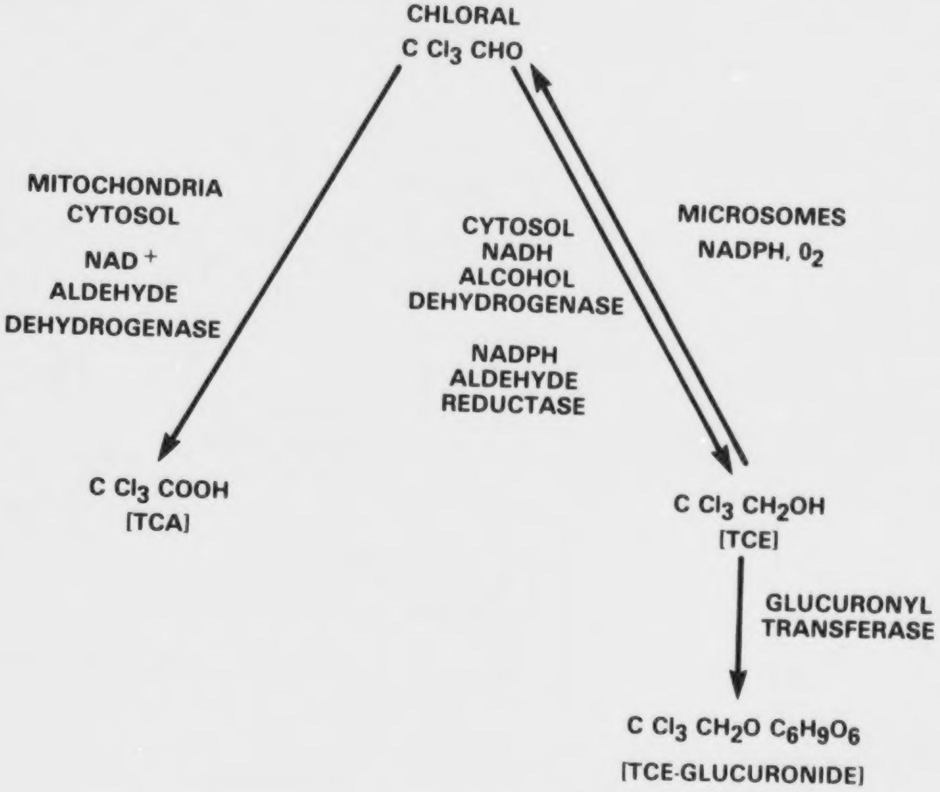


Figure 4-11. Metabolism of chloral hydrate.

Source: Modified from Ikeda et al. (1980).

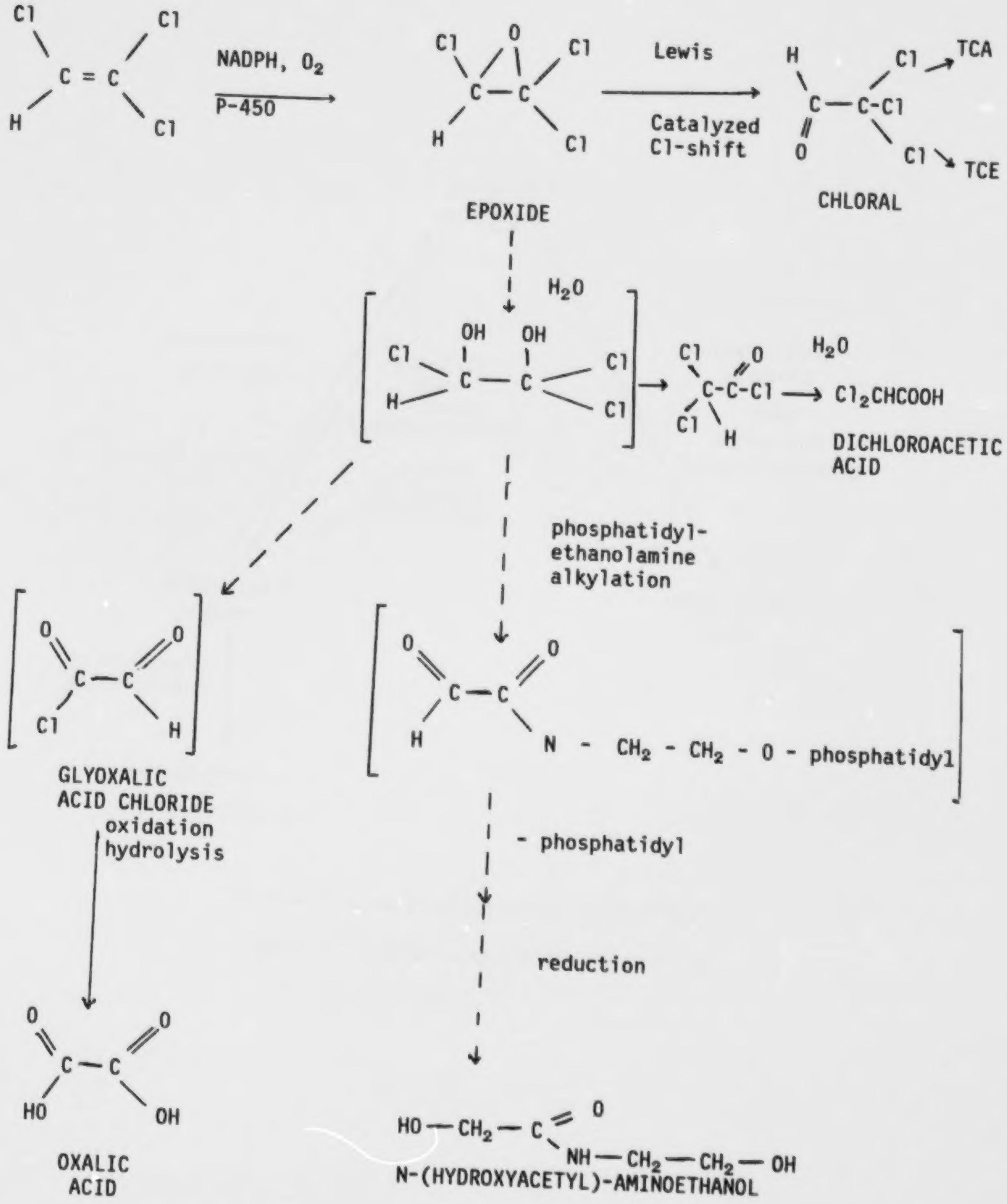


Figure 4-12. Postulated scheme for the metabolism of TCI based on urinary metabolite profiles of rats and mice. From Dekant et al., 1984. Suggested pathways are indicated by dashed arrows.

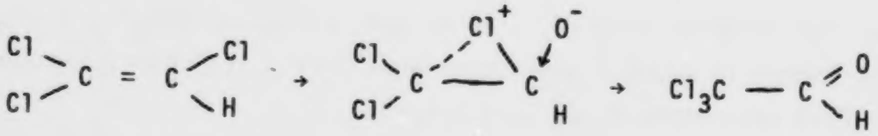
pools was observed in the conversion of ^{36}Cl-TCI to its end products TCE and TCA. Microsomal P_{450}-mediated chloral formation from TCI has been repeatedly confirmed (Ikeda et al., 1980, Miller and Guengerich, 1982) and the formation of the epoxide (1,1,2-trichloroethene oxide) has also been directly demonstrated in liver microsomal preparations by trapping with p-nitrobenzyl pyridine (Miller and Guengerich, 1982). In addition, a specific P_{450} binding spectrum (Type I) in liver microsomes of rats and rabbits has been observed after addition of TCI or TCI-epoxide (Kelley and Brown, 1974; Uehleke et al., 1977b; Pelkonen and Vaino, 1975). TCI also competitively inhibits the metabolism of hexobarbital and aniline by microsomal fractions (Kelley and Brown, 1974), and the magnitude of the binding and chloral formation is enhanced with microsomes from animals pretreated with phenobarbital but not 3-methylcholanthrene (Pelkonen and Vaino, 1975; Leibman and McAllister, 1967). However, the question arises whether the epoxide is an obligatory intermediate in the formation of chloral (Miller and Guengerich, 1982).

Attempts have been made to demonstrate chloral as a metabolite of TCI-oxide. TCI-oxide has been synthesized and its reactions studied under a variety of conditions ($t_{½}$ ~ 1.3 minutes in water at pH 7.4, 37°C) Bonse et al., 1975; Kline and Van Duuren, 1977; Henschler et al., 1979; Miller and Guengerich, 1982). Henschler et al. (1979) reported that, under physiological conditions (Tris buffer, pH 7.4), TCI-oxide rearranged to dichloroacetic acid, CO, formic acid, and glyoxylic acid, but not to chloral. Entirely similar results were observed by Miller and Guengerich (1982). However, Bonse and Henschler (1976) demonstrated that the TCI oxide can be forced to rearrange to chloral by the catalytic action of Lewis acids ($FeCl_3$, $AlCl_3$, BF_3), and Henschler and co-workers proposed that the formation of chloral in vivo could therefore be due to catalytic action of the iron of P_{450} in the trivalent form at the hydrophobic microsomal site of formation of the oxide (Figure 4-2). Although Miller and Guengerich (1982) found that excess ferric iron salts catalyzed the rearrangement of TCI-oxide to chloral in nonaqueous solutions (CH_2Cl_2 or CH_3CN), chloral was not formed in aqueous media even when iron salts, ferriprotoporphyrin IX or purified cytochrome P_{450}, were present.

In studies with rat or mouse liver microsome fortified with NADPH and O_2 (or a reconstituted P_{450} enzyme system), Miller and Guengerich (1982) found that TCI was metabolized to chloral, TCI-oxide, glyoxylic acid and CO. Traylor et al. (1977) also found TCI converted to CO in microsomal incubations.

Addition of TCI-oxide to these systems did not produce chloral, but the oxide disappeared with a $t_{\frac{1}{2}} \sim 17$ seconds or, in the presence of rat liver epoxide hydrolase, 9 seconds. On the basis of the reaction kinetics of formation of chloral and disappearance of TCI-oxide in microsomal preparations, Miller and Guengerich (1982) proposed that TCI-oxide was not an obligatory intermediate step in the formation of chloral. Alternatively, these investigators postulated an oxygenated TCI-P_{450} transition state leading to two products: chloral (with chloride migration) and a separate epoxide formation, as shown in Figure 4-11. The working scheme for the metabolism of TCI as presented in Figure 4-11 accounts for all the microsomal metabolites of TCI. Miller and Guengerich (1982) excluded the likelihood of a rearrangement of TCI-oxide or of TCI glycol to chloral, because while rearrangement to chloral occurred in non-aqueous media in the presence of excess ferric iron salt, the incubation of TCI-oxide with purified P_{450} did not result in rearrangement to chloral. Since _in vivo_ chloral is the primary metabolite of TCI metabolism, then in view of Miller and Guengerich's (1982) postulate, the P_{450}-mediated reaction to chloral may be preferential. The possibility of TCI-epoxide being a secondary, minor product in the metabolism of TCI may account for the lesser mutagenic and oncogenic potential of TCI in comparison to other halogenated ethylenes (Bolt et al., 1982; Dekant et al., 1984).

Van Dyke (1977) and Van Dyke and Chenoweth (1965) also proposed a direct microsomal oxidation to chloral. TCI conversion to chloral was postulated to involve a chloronium ion transition intermediate state in concert with oxidation, presumably by cytochrome P_{450} system. As illustrated, the mechanism does not require an epoxide intermediate. The oxidative attack forces chloride migration to the adjacent carbon if possible, as in the case of TCI; if not, then the chlorine bonded to the carbon, which is oxidatively attacked, is released (dechlorination).



This mechanism also results in "reactive" intermediates which can covalently bind to cell constituents.

4.4.3.2 Metabolism of chloral hydrate--The enzymatic pathways for the biotransformation of chloral hydrate formed from TCI exposure, to the plasma and urinary metabolites TCE, TCE-glucuronide, and TCA, have received less attention in recent years than the mechanism of chloral formation. Current knowledge of the enzymatic pathways of chloral hydrate biotransformation is outlined in Figure 4-12. Chloral hydrate is very rapidly metabolized *in vivo* with a half-life of only a few minutes (Marshall and Owens, 1954; Breimer et al., 1974; Cole et al., 1975).

A number of investigators have concluded from *in vitro* studies that chloral hydrate is a substrate for alcohol dehydrogenase purified from horse liver and rat liver cytosol fractions (Butler, 1948, 1949; Marshall and Owens, 1954; Friedman and Cooper, 1960; Sellers et al., 1972b). By this reaction, with NADH as the required coenzyme, chloral hydrate is reduced to TCE (K_m, 2.7×10^{-3}M, horse enzyme). The reaction is essentially unidirectional, since conversion back to chloral hydrate has not been observed with this enzyme (Sellers et al., 1972b; Owens and Marshall, 1955; Friedman and Cooper, 1960). Tabakoff et al. (1974) studied the reduction of chloral to TCE and postulated the presence of enzymes in rat liver cytosol and the presence of enzymes other than alcohol dehydrogenase that are capable of reducing chloral. Ikeda et al. (1980) demonstrated at least two NADPH-dependent enzymes to be present in rat liver cytosol (in addition to NADH-dependent alcohol dehydrogenase) with a K_m of 6.0×10^{-3}M. These investigators also found that rat liver microsomes in the presence of NADPH and O_2 oxidized TCE to chloral similarly to the oxidation of methanol and ethanol to acetaldehyde (Liebler and DiCarli, 1969).

Human red cells also reduce chloral hydrate to TCE (K_m, 4.0×10^{-4}M) (Sellers et al., 1972b). The efficiency of red cells and their relatively large mass may explain the nearly undetectable levels of chloral hydrate in plasma and urine after exposure to TCI, while TCE is prominent in plasma and urine. TCE-glucuronide present in plasma and urine is accepted as resulting from conjugation by hepatic microsomal glucuronyl transferase.

The origin of TCA as a plasma and urinary metabolite of chloral hydrate is not defined as clearly. The most likely reaction for the oxidation of chloral hydrate to TCA would involve the well-known enzyme acetaldehyde dehydrogenase. However, chloral hydrate has been reported not to be a substrate for human acetaldehyde dehydrogenase (Kraemer and Deitrich, 1968; Blair and Bodley, 1969; Sellers et al., 1972b). Cooper and Friedman (1958) obtained from rabbit liver acetone powder a partially purified form of an NAD-dependent "chloral hydrate dehydrogenase" which was substrate-specific for chloral hydrate. Acetaldehyde was not a substrate but rather a markedly effective inhibitor. Chloral dehydrogenase has not been identified in any other species even though the rabbit, in comparison with the rat or man, poorly converts TCI to TCA. Furthermore, Cooper and Friedman (1958) reported that their enzyme was not inhibited by disulfiram. However, orally administered disulfiram is known to markedly decrease excretion of TCA and TCE after TCI exposure to man (Bartonicek and Teisinger, 1962) and to dogs and rats (Forssman, 1953, cited in Soucek and Vlachova, 1960).

In contrast to earlier studies on aldehyde dehydrogenase that found most of this enzyme activity in the cytosol (Büttner, 1965), more recent studies show that about 80 percent of total activity resides in the mitochondrial and microsomal cellular fractions. Tottmar et al. (1973) described at least two different aldehyde dehydrogenases (NAD and NADP-dependent) in rat liver, localized respectively in mitochondria and microsomes. Grunett (1973) found the NAD-dependent mitochondrial enzyme to have a broad substrate specificity, but chloral hydrate was not a substrate. However, Ikeda et al. (1980) found that an aldehyde dehydrogenase prepared from rat liver mitochondria (NAD-dependent) converted chloral to TCA (K_m, 62.5×10^{-3}M). Furthermore, rat liver mitochondria had the highest specific activity for TCA formation, cytosol less, but the microsomal fraction contributed little to the formation of TCA.

Present evidence indicates that the liver is the principal organ contributing to the metabolism of TCI to TCE and TCA. For example, Bonse et al. (1975) showed that TCI perfused through the isolated rat liver led to the appearance of TCA, TCE, and chloral in the post perfusate. However, other tissues may also contribute significantly. Dalbey and Bingham (1978) have demonstrated the metabolism of TCI by the isolated perfused lungs of rats and guinea pigs to TCE and TCA, and pretreatment of rats with phenobarbital increased the lung metabolism to TCE. Tabakoff et al. (1974) found that rat brain cytosol was capable of reducing chloral to TCE. The conversion of chloral in the presence of NADPH was 5 times faster than with NADH, the co-factor for alcohol dehydrogenase. As noted above, human red blood cells actively reduce chloral to TCE.

4.4.3.3 Minor Metabolites and Pathways--For rats and mice exposed to TCI, several metabolites (CO_2, oxalic acid, dichloroacetic acid, HAAE; Tables 4-11 and 4-12) other than TCA, TCE, and TCE-glucuronide have been identified in exhaled air, urine, feces, or bile. Some of these minor metabolites have also been identified in human urine (dichloroacetic acid, HAAE). Together, these metabolites represent less than 15 percent of the TCI dose in rodents (Table 4-12).

Dekant et al. (1984) have suggested that the appearance of small amounts of dichloroacetic acid in the urine of rats and mice (Table 4-12), and possibly of man (Table 4-11), stems from a limited rate of conversion of TCI-oxide to chloral with escape of the epoxide from the hydrophobic environment of P_{450}, and subsequent decomposition in hydrophilic conditions to dichloroacetic acid and other compounds.

CO_2 from TCI metabolism has been postulated by Miller and Guengerich (1982) to originate from hydrolytic rearrangement or cleavage of the epoxide to formyl chloride and glyoxalic acid chloride with hydrolysis to formic acid and glyoxalic acid, respectively (Figure 4-11). These two metabolites, entering the endogenous one- and two-carbon pools, are then metabolized further to CO_2. Green and Prout (1984) have shown that CO_2 may also be the product of decarboxylation of TCA and dichloroacetic acid. These investigators have administered ^{14}C-TCI to rats and mice by gavage or intravenously and demonstrated that 7 to 15 percent of the dose was recovered in exhaled breath as $^{14}CO_2$ within 24 hours. Green and Prout suggest the formation of a coenzyme A ester of TCA followed by degradation to oxalyl CoA and subsequent release of two moles of CO_2.

Dekant et al. (1984) have recently identified two new metabolites of TCI, oxalic acid and HAAE, in the urine of rats and mice. These investigators suggest that oxalic acid may be formed as the end-product of enzymatic or non-enzymatic cleavage of TCI-oxide as shown in Figure 4-13. Hydrolytic ring opening produces the glycol which is expected to undergo spontaneous elimination of two molecules of HCl to form glyoxalic acid chloride with subsequent hydrolysis to glyoxalic acid, which then is oxidized further to oxalic acid. The spontaneous formation of glyoxalic acid from TCI-oxide decomposition in aqueous solution has been amply demonstrated (Table 4-12). HAAE also had not been identified previously as a metabolite of TCI. Dekant et al. suggest that TCI-oxide may interact with ethanolamine or phosphatidyl-ethanolamine in cell membranes to produce HAAE as indicated in Figure 4-13. The minor metabolites of TCI may occur as follows: if it is assumed that the conversion of TCI-oxide to chloral is a rate-limiting step in the P_{450}-mediated metabolism of TCI, then at high substrate concentrations of TCI an accumulation of TCI-oxide may occur and activate alternative pathways leading to the formation of metabolites other than chloral.

4.4.4 Metabolism and Covalent Binding

The extent of irreversible or covalent binding of reactive intermediates from the metabolism of TCI to cellular macromolecules (protein, RNA, DNA) has been extensively investigated, both *in vitro* and *in vivo*, as a theoretical basis for evaluating the mutagenic and carcinogenic potential of TCI (Bolt et al., 1982). The microsomal oxidative metabolism of TCI to chloral, as outlined in Figures 4-11 and 4-13, suggest the possible reactive metabolites that may result in covalent binding include chloral, TCI-epoxide, dichloroacetyl chloride and formyl chloride. Chloroform, a putative metabolite as yet of indeterminate importance in TCI metabolism, is known to generate covalent binding (Davidson et al., 1982). The derivative metabolites of chloral (TCE, TCE-glucuronide, TCA) have not been implicated in covalent binding. Primary concern accrues to the highly reactive epoxide formed in TCI metabolism.

Henschler and coworkers (Bonse et al., 1975; Greim et al., 1975; Bonse and Henschler, 1976; Henschler and Bonse, 1979) suggested that all chlorinated ethylenes are initially transformed by microsomal P_{450} systems to epoxide intermediates which are subsequently "detoxified" by molecular rearrangement (in the case of TCI to chloral). Further, reactivities, and hence, toxicities of individual epoxide intermediates depend on the type of chlorine substitutions, in that symmetric substitution renders the epoxide relatively stable and not mutagenic, but asymmetric substitution causes unstable and therefore mutagenic epoxides. To explain the lower-than-predicted mutagenic and carcinogenic potential of TCI (Chapter 7), these investigators suggest that TCI is an exception to this general rule, because though its epoxide is asymmetrically substituted and reactive, it immediately upon formation rearranges within the hydrophobic premises of the P_{450} site to the less reactive chloral; thus the epoxide is isolated from reaction with cellular macromolecules and protected from aqueous spontaneous decomposition to other reactive metabolites (dichloroacetyl chloride, formyl chloride) (Henschler et al., 1979). Alternatively, Miller and Guengerich (1982) have provided evidence that the epoxide is not an obligatory intermediate for chloral formation, and that, indeed, the two are separate P_{450} oxidative products with chloral production four- to five-fold greater than the epoxide. Furthermore, they observed a four- to five-fold greater production of chloral plus epoxide from mouse microsomes than from rat microsomes.

4.4.4.1 In Vitro Binding. Table 4-13 lists investigators who have demonstrated covalent binding of TCI to microsomal protein nucleic acids and lipids *in vitro*. In many of these experiments, *in vitro* covalent binding from TCI microsomal metabolism was decreased by animal pretreatment with SKF-525A (which inhibits P_{450} metabolism) or enhanced by phenobarbital and other inducers of the P_{450} system. Binding was also decreased by addition of an inhibitor of epoxide hydratase (trichloropropane epoxide) and by the addition of competing nucleophiles such as reduced glutathione, imidazole or mercaptoethanol, indicating the importance of cellular sulfhydryl groups and glutathione specifically for providing a "detoxification mechanism" for reactive intermediates from TCI metabolism. These experiments provide ample evidence that TCI is metabolized *in vitro* to reactive products by P_{450} systems. Metabolism and covalent binding of reaction products occurs with liver microsomes, the major site of metabolism, but also with microsomes from kidney, stomach, and lung (Banerjee and Van Duuren, 1978). Mouse microsomes are two- to four-fold more active in metabolizing TCI to reactive intermediates than rat microsomes, and males greater than females (Uehleke and Poplawski-Tabarelli, 1977; Banerjee and Van Duuren, 1978). DiRenzo et al. (1982a) found that aging rats (27 months old) have less capacity for microsomal metabolism, as reflected by covalent binding of TCI, than either adult (11 months old) or young (4 months) rats. These investigators have also compared the rat hepatic microsomal covalent binding to added DNA for a series of aliphatic halides, including TCI. The degree to which TCI and other aliphatic halides form a DNA-adduct is summarized in Table 4-14. TCI was bioactivated and covalently bound to DNA at a level of 0.36 nmol/mg DNA, or fifth in the series of 10 compounds. Binding was confirmed by sedimentation of the DNA-adduct in a cesium gradient and Sephadex LH-20 chromatography of the nucleotides, although structural identification of modified DNA bases was not made.

4.4.4.2 In Vivo Binding. A number of investigators have determined the extent of irreversible binding to cellular macromolecules following the administration of TCI to rats and mice. Bolt and Filser (1977) exposed rats (male Wistar) for 5 hours in a closed system to initial concentrations of 100 and 1000 ppm (538 and 5380 mg/m^3) ^{14}C-TCI and determined protein-bound metabolites in

TABLE 4-13. *IN VITRO* COVALENT BINDING OF TCI

Investigator	System	Macromolecules covalently bound	Additions
Van Duuren and Banerjee (1976)	rat liver microsomes	microsomal protein	SKF-525A or 7,8 benzoflavone- ↓ binding; GSH, methylmercaptoimidazole, mercaptoethanol, urea-↓ binding; epoxide hydratase inhibitor (trichloropropane epoxide) ↑ binding; pretreatment with phenobarbital- ↑ binding
Bolt and Filser (1977) Bolt et al. (1977)	rat liver microsomes	albumin, polylysine	GSH, ↓ binding
Uehleke and Poplawski-Tabarelli (1977)	rat and mice liver microsomes	microsomal protein lipid	Mouse > rat; pretreatment with phenobarbital, ↑ binding
Allemand et al. (1978)	rat liver microsomes	microsomal protein	GSH or piperonyl butoxide - ↓ binding; pretreatment of animals with phenobarbital - ↑ binding
Banerjee and Van Duuren (1978)	Mice (B_6C_3F) and rat (Osborne-Mendel) microsomes	microsomal protein	Mice > rats, males > females; microsomes from all organs bind equally
		Salmon sperm DNA	Male > female enhanced by phenobarbital and 3-methylcholanthrene admin; enhanced by addition of epoxide hydratase inhibitor (trichloropropane epoxide)
Laib et al. (1979)	rat liver microsomes	yeast RNA microsomal protein	VC > TCI TCI > VC
DiRenzo et al. (1982a)	rat liver microsomes	calf thymus DNA	Rats pretreated with phenobarbital
	rat liver microsomes young (4 mo) vs adult (11 mo) vs aged (27 mo)	microsomal protein	↓ P_{450} system in aged; ↓ binding; pretreatment with phenobarbital abolishes difference

TABLE 4-14. MICROSOMAL BIOACTIVATION AND COVALENT BINDING OF ALIPHATIC HALIDES TO CALF THYMUS DNA

Aliphatic halides	Binding to DNA (nmol/mg/h ± SD)
1,2-Dibromoethane	0.52 ± 0.14 (6)
Bromotrichloromethane	0.51 ± 0.18 (6)
Chloroform	0.46 ± 0.13 (6)
Carbon tetrachloride	0.39 ± 0.08 (6)
Trichloroethylene	0.36 ± 0.14 (7)
1,1,2-Trichloroethane	0.35 ± 0.07 (7)
Dichloromethane	0.11 ± 0.05 (5)
Halothane	0.08 ± 0.01 (6)
1,2-Dichloroethane	0.06 ± 0.02 (6)
1,1,1-Trichloroethane	0.05 ± 0.01 (3)

^{14}C-labeled aliphatic halides (1 mM) were incubated with hepatic microsomes. Carbon tetrachloride, bromotrichloromethane and halothane were incubated under an N_2 atmosphere, and all other incubations were under an O_2 atmosphere.

Source: DiRenzo et al. (1982b).

various organs. The irreversibly bound metabolites were calculated as a percentage of that amount of TCI that was taken up by the animals in 5 hours. Radioactivity following exposure was mainly concentrated in the liver (0.77 - 0.88 percent dose), the primary organ of metabolism, and in kidneys (0.37 - 0.39 percent dose), the organ of primary excretion. Lesser amounts were found in lung, spleen, small intestine, and muscle. No major differences in percentage of the dose bound versus the different initial atmospheric concentrations of TCI were observed. However, the extent of binding in liver and kidney for TCI was less than for vinyl chloride and approximated that observed with carbon tetrachloride.

Uehleke and Poplawski-Tabarelli (1977) determined in vivo binding for mice (NMRI) following i.p. injections of ^{14}C-TCI (0.2 µCi/10 µmole in 5 µl of peanut oil/g b.w.). Binding to liver microsomes, mitochondria, and cytosol protein was measured at 2, 6, 12, and 24 hours after injection. Peak binding occurred after 6 hours (8, 4 and 1.8 nmol/mg protein) respectively, and thereafter declined slowly. When the same dose of ^{14}C-TCI was given by gavage at the 6-hours time, binding to microsomal protein was found to be only 5.4 nmol/mg protein. ^{14}C-TCI was also bound to hepatic lipids; similarly to protein binding, 6 hours after i.p. dosage, the highest concentrations were 9.4 nmol/mg of endoplasmic fractions of livers and 5.6 nmol/mg in mitochondrial lipids of the liver.

Allemand et al. (1978) investigated the metabolic activation of TCI and irreversible binding in Sprague-Dawley rats after ^{14}C-TCI (100 µmol, 100 µCi) was administered i.p. in 200 µl of methanol to nonpretreated and to phenobarbital-pretreated rats; the rats were sacrificed 4 hours later. Trace amounts of irreversibly bound radioactivity were found on muscle proteins (3.1 mmol equiv./g tissue), while 40 times more was found on liver proteins (117 nmol equiv./g tissue). Pretreatment of the animals with phenobarbital did not significantly increase muscle binding but increased liver binding by 46 percent. Furthermore, TCI reduced hepatic glutathione levels 61 percent 4 hours after administration, confirming similar observations by Moslen et al. (1977b).

Parchman and Magee (1982) compared _in vivo_ covalent binding of TCI metabolites to liver protein and DNA in B6C3F1 male mice and Sprague-Dawley male rats. Rats were injected i.p. with 10, 100, 500, and 1000 mg/kg b.w. of ^{14}C-TCI in corn oil (each animal received 160 µCi/kg of radioactivity). After 6 hours, liver "cytoplasm" and "nucleus" fractions contained 2.5 to 6.1 and 0.3 to 3.9 percent of dose radioactivity, respectively. Radioactivity was detected in DNA isolated from the nucleus fraction and purified by cesium chloride centrifugation and extraction with phenol, isoamyl alcohol, and chloroform. However, the level of radioactivity was extremely low (35 cpm/mg DNA) and contamination with labeled protein could not be entirely ruled out. Identification of DNA adducts by hydrolysis followed by HPLC of nucleotides was not successful. Similar results were obtained with mice injected i.p. with 10 and 250 mg/kg ^{14}C-TCI (160 µCi/kg b.w.) and sacrificed 6 hours later. Hepatic DNA contained only 51 cpm/mg for the lower dose and 283 cpm/mg for the higher dose. Compared to DNA binding by dimethylnitrosamine (DMN) under identical conditions, the carcinogen-binding index (Lutz, 1979) calculated for TCI was only 2, as compared to 2045 for DMN. These investigators concluded that the ability of TCI to interact with DNA _in vivo_ was very slight.

Stott et al. (1982) investigated covalent binding of TCI in male B6C3F1 mice and male Osborne-Mendel rats, the rodent strains used for carcinogenicity testing of TCI by the National Cancer Institute (NCI) (NCI, 1976). The animals were exposed to 10 or 600 ppm (54 or 3228 mg/m^3) of ^{14}C-TCI (11.3 mCi/nmol) for 6 hours. The mice metabolized more inhaled TCI than rats at low and high exposure levels (123 and 260 percent, respectively). The amount of hepatic and renal covalent binding was three-fold higher in mice than for the high dose (Table 4-15) and four-fold greater for liver than kidney. Mice (4) were also dosed with 1200 mg/kg of ^{14}C-TCI with relatively high specific activity (2.4 mCi/nmol) and a maximum estimate of a liver DNA alkylation level of 0.62 ± 0.42 alkylations/10^6 nucleotides was observed after isolation and separation by HPLC (Table 4-16). However, no chemical identification of possible nucleotide adducts was made. Maximum covalent binding index observed was 0.12, or one-twentieth of that estimated by Parchman and Magee (1982), and many orders of magnitude less than that for known carcinogens such as DMN, methylnitrosurea, etc. (footnote, Table 4-15). These results are in accord with the conclusion of Parchman and Magee (1982) that the ability of TCI to interact with DNA _in vivo_ is very slight.

Stott et al. (1982) also chronically administered to male B6C3F1 mice (by gavage) 2400 mg TCI/kg/day for 3 days. Localized cell necrosis, enhanced DNA synthesis, and centrilobular hepatocellular swelling were observed. With prolonged exposure (3 weeks), the primary response observed was dose-related centrilobular hepatocellular swelling and the occurrence of mineralized cells. In Osborne-Mendel male rats, a maximum tolerated dose of 1100 mg/kg/day led to increased liver weight and elevated DNA synthesis not coupled to histopathology, but consistent with a simple increase in the number of normal hepatocytes. In view of the weak genotoxic potential of TCI, as indicated by only slight DNA covalent binding and weak hepatotoxicity in mice, these investigators suggest an epigenetic mechanism for the tumorigenic action of TCI observed in this species only in the NCI carcinogenicity bioassay (NCI, 1976). They implicate also the greater covalent protein binding (three-fold) that occurs in the mouse versus the rat from the three-fold greater total metabolism of TCI per kilogram body weight.

4.4.5 TCI Metabolism: Drug and Other Interactions

A number of commonly used drugs might be expected to modify the extent of metabolism of TCI during human exposure. The converse may also occur, resulting in important modifications of the therapeutic action of drugs. Although largely undocumented in man, the induction of hepatic microsomal mixed-function oxidate system by drugs taken for therapeutic reasons or by exposure to certain environmental chemicals (e.g., barbiturates, PCBs, etc.), may bring about an increased rate of TCI metabolism. Numerous _in vivo_ and _in vitro_ experimental

TABLE 4-15. HEPATIC AND RENAL MACROMOLECULAR BINDING OF 1,1,2 (UL-^{14}C) METABOLITE IN MALE B6C3F1 MICE AND OSBORNE-MENDEL RATS EXPOSED TO 10 OR 600 PPM FOR 6 HOURS

Exposure (ppm)	Tissue	Test animal	Binding (pmol-eq(^{14}C) TCI/µg protein) ± SD	Ratio (mouse/rat)
10	Liver	Mouse Rat	0.318 (0.004) 0.268 (0.013)	1.2
	Kidney	Mouse Rat	0.168 (0.014) 0.155 (0.007)	1.1
600	Liver	Mouse Rat	20.4 (2.49) 4.72 (0.415)	4.3
	Kidney	Mouse Rat	5.06 (0.667) 1.77 (0.200)	3.0

Source: Stott et al., 1982.

TABLE 4-16. *IN VIVO* ALKYLATION OF HEPATIC DNA BY 1,1,2(UL-^{14}C) TCI IN MALE B6C3F1 MICE DOSED WITH 1200 MG/KG (^{14}C) TCI BY GAVAGE

Animal	Detection limit (alk/10^4)	Non-C_1 dpm[a] > 2 Sigma error	Maximum alkylations 10^4 nucleotides[b]	Maximum CBI[c]
1	0.12	18.0	0.50	0.055
2	0.52	ND[d]	--	--
3	0.15	7.1	0.27	0.030
4	0.18	26.6	1.1	0.120

[a] dpm not associated on HPLC analysis with normal DNA bases from normal metabolic incorporation and not attributable to counting error (i.e., mean background dpm + 2 SD).

[b] "Maximum" alkylations possible due to the relatively large amount of dpm associated with protein binding and metabolic incorporation into normal bases.

[c] Covalent binding index (Lutz, 1979). Comparative hepatic CBI for dimethylnitrosamine, diethylnitrosamine, methylnitrosourea, and aflatoxin B-1 are approximately 5500, 125, 640, 17,000, respectively. CBI = (µmol adduct/mol dN) (nmol/kg dose).

[d] None detected.

Source: Stott et al. (1982).

studies with animals pretreated with microsomal inducers have demonstrated an enhanced metabolism of TCI (Leibman and McAllister, 1967; Carlson, 1974; Moslen et al., 1977a,b; Pessayre et al., 1979). Conversely, TCI competitively inhibits the metabolism of barbiturates, producing exaggerated effects of these drugs (Kelley and Brown, 1974).

4.4.5.1 Disulfiram. A drug interaction has been shown to occur in persons exposed to TCI who are also on a therapeutic regimen of disulfiram for alcoholism. Disulfiram inhibits the oxidation of acetaldehyde by competing with NAD for active sites on aldehyde dehydrogenase. Bartonicek and Teisinger (1962) showed that disulfiram markedly inhibits the terminal enzymatic steps of TCI metabolism in man. Since these steps, which involve the conversion of chloral hydrate to TCE and TCA (Figure 4-12), can be considered detoxification mechanisms, disulfiram has the potential of enhancing TCI and chloral hydrate toxicities.

4.4.5.2 Alcohol. Sellers et al. (1972b) investigated the interaction of ethanol and chloral hydrate metabolisms. These investigators observed that concomitant administration of alcohol and chloral hydrate in man exacerbated the side effects of chloral hydrate exposure (vasodilatation, tachycardia, facial flushing, headache and hypotension). Their patients also exhibited impairment of auditory function and of performance of complex motor tasks. Similar effects have been observed during acute and chronic TCI exposure and concomitant ingestion of alcohol (Muller et al., 1975). Stewart et al. (1974) have described the phenomenon of "degreasers" flush (facial and upper extremity skin vasodilatation) and determined that this effect of alcohol interaction with TCI persists after cessation of exposure. This intolerance to alcohol syndrome is ascribed to mutual inhibition of TCI and alcohol metabolism producing increased plasma ethanol and TCE concentrations from competitive inhibition of aldehyde dehydrogenase (Sellers et al., 1972a,b; Stewart et al., 1974).

Muller et al. (1975) specifically investigated the interaction of TCI and alcohol in human volunteers. After the volunteers inhaled TCI (50 ppm) for 6 hours per day for 5 days, ethanol ingestion inhibited TCI metabolism to TCE and TCA by 40 percent. Blood TCI concentration increased more than two-fold. This latter observation suggested that alcohol also inhibited, by competition, the microsomal oxidation of TCI to chloral hydrate. These investigators proposed that the intolerance syndrome from combined exposure to TCI and ethanol was due to increased accumulation of TCI in the CNS, resulting from depression of TCI oxidation. From experiments in rats, Nakanishi et al. (1978) found that ethanol ingested after TCI exposure increased blood acetaldehyde levels three- to four-fold. They suggest that TCI inhibits acetaldehyde dehydrogenase, and the resulting increase of blood levels of acetaldehyde is responsible for the intolerance syndrome.

White and Carlson (1981), working with rabbits, observed that acute ethanol administration (1 g/kg i.v. or p.o. 30 minutes prior to TCI exposure, 6000 ppm; 32280 mg/m^3) decreased TCI metabolism, as indicated by increased peak blood levels of TCI (22 percent), decreased peak blood levels of TCE (50 percent) and decreased blood levels of TCA (99 percent). Also, the zero-order metabolism of alcohol itself was decreased 25 percent by TCI exposure (blood ethanol disappearance rate, -0.707 control versus -0.539 exposed, mg/dl/min). Furthermore, rabbits treated with ethanol exhibited markedly increased susceptibility to the development of TCI-induced cardiac arrhythmias, provoked by epinephrine. The results of this ethanol study in rabbits confirm those of the human studies by Muller et al. (1975).

In contrast to these studies, Sato et al. (1981) found that an acute ethanol dose to rats (4 g/kg, 40 percent solution intragastric) significantly lowered the blood concentration decay curve of TCI resulting from a subsequent (16 hours later) 400 ppm (2152 mg/m^3) for 4-hr exposure to TCI. Furthermore, ethanol-treated rats excreted in urine much greater amounts (three-fold) of total trichloro compounds (TCE and TCA) than untreated rats. These findings would indicate that a single large dose of ethanol can accelerate the _in vivo_ metabolism of TCI. When ethanol (4 g/kg) was given to rats and liver microsomes were prepared and tested for their rate of metabolism of TCI, an enhancement of TCI metabolic rate was found, with the greatest increase (two-fold) occurring 16 hours after ethanol dosing. At this time, almost no blood ethanol was detected and no increase occurred in microsomal protein and P_{450} liver contents. Ethanol addition directly to the TCI microsomal incubations inhibited TCI metabolism, thus demonstrating that ethanol was a competitor of TCI metabolism. These investigators suggest that ethanol, when taken _in vivo_, can exert a dual effect on drug-metabolizing enzymes, i.e., both inhibition and stimulation of TCI metabolism. When present in an early period at high blood levels, ethanol inhibits metabolism of TCI; but ethanol also increases metabolic potential of the enzymes so that 16 to 18 hours later, when ethanol blood level has dissipated, the microsomal enzymes have an increased capacity to metabolize TCI.

4.4.5.3 Warfarin. Sellers and Koch-Weser (1970) observed a potentiation of the anticoagulant effect of Warfarin in patients after chloral hydrate ingestion. This increased potential for bleeding appeared to result from displacement of Warfarin from plasma protein binding sites by the chloral hydrate metabolite, TCA. Considering the significant amounts of TCA formed after TCI exposure and the long half-time of excretion of TCA, it is likely that TCI may potentiate the effects of many other drugs which normally bind to the same plasma protein sites as TCA.

4.4.5.4 Diet and Age. Nakajima et al. (1982) have explored the effect of diet on the hepatic microsomal metabolism of TCI and other chlorinated hydrocarbons. These investigators found that hepatic microsomes from rats fed for 3 weeks on an isocaloric diet deficient in carbohydrate (sucrose) had an increased capacity (two and one half-fold) to metabolize TCI and other chlorinated hydrocarbons. Varying protein content of the diet produced only a negligible influence on enzyme activity. Microsomal protein and P_{450} hepatic content (per g liver) were not significantly changed, although liver weight was slightly decreased. They suggest from their findings that a low-carbohydrate diet produces enhanced P_{450} system activity. This concept, according to these investigators, is not in conflict with the widely held belief that a high-protein diet enhances this activity, because high-protein diets are usually low in carbohydrate to maintain isocaloric conditions. Thus, a diet rich in carbohydrate may offer significant protection against the hepatic toxicity of chlorinated compounds by decelerating their metabolism.

DiRenzo et al. (1982a) have recently shown that the hepatic microsomal capacity to metabolize TCI and other chlorinated hydrocarbons is influenced by chronological age. These investigators isolated hepatic microsomes from young (4 months), adult (11 months) and aging (27 months) Fischer 344 rats. Biotransformation and subsequent covalent binding of labeled TCI and other compounds was studied. Levels of microsomal protein and lipid binding increased slightly between 4 and 11 months, but decreased 50 to 75 percent in the 27-month-old animals, as compared to young adults. Senescent rats were also found to have significantly less hepatic P_{450} (-35 percent), NADPH cytochrome C reductase (-31 percent), and ethylmorphine N-demethylase activity (-43 percent). Pretreatment with phenobarbital, however, abolished these deficiencies of aging rats. These investigators suggest a reduced metabolic capacity as a function of age, which results in a decrease in bioactivation of the aliphatic halides, and hence, possible differences in toxicity in the elderly from xenobiotics requiring bioactivation.

4.4.6 Metabolism and Cellular Toxicity

The metabolism of TCI has been suggested as a possible vector for the hepatotoxicity and nephrotoxicity associated with excess human exposure to this haloalkene (James, 1963; Defalque, 1961; Smith, 1966; Litt and Cohen, 1969; Baerg and Kimberg, 1970). Experimentally, TCI has been shown to be hepatotoxic in dogs and rats (Orth and Gillespie, 1945; Klaassen and Plaa, 1967; Cornish and Adefuin, 1966; Carlson, 1974; Cornish et al., 1977; Reynolds and Moslen, 1977). Reynolds and Moslen (1977) and Henschler and Bonse (1979), on physicochemical and biological correlative bases, have proposed that unsymmetric chlorinated ethylenes like TCI are more hepatotoxic than symmetric ethylenes as perchloroethylene and more so than vinyl chloride. The mechanism for TCI hepatotoxicity is not definitely established, but many investigators have proposed that the observed centrilobular cellular necrosis results from the microsomal mixed-function oxidase system formation of chemically "reactive" metabolites which react with and bind to tissue macromolecules and cause intracellular damage (Pessayre et al., 1979; Allemand et al., 1978; Reynolds and Moslen, 1977). Figures 4-11 and 4-13 indicate that these reactive metabolites may include TCI epoxide, dichloroacetyl chloride, and formyl chloride (Section 4.4.4). Chloral, chloral hydrate, and TCE, as ordinarily used hypnotics, are not associated with significant hepatotoxicity (Goodman and Gilman, 1975).

Buben and O'Flaherty (1984) have recently provided convincing evidence that the hepatotoxicity of TCI is directly proportional to the extent to which TCI is metabolized. These investigators administered to male Swiss-Cox mice by gavage TCI in corn oil vehicle for 6 weeks (5 days/week), at dose levels of 100 to 3200 mg/kg. Metabolism was measured by the amount of urinary metabolites (TCA, TCE, and TCE glucuronide) excreted per day (24-hr urine collections). Indices of hepatotoxicity measured in the dosage groups were increases in liver weight, decreases in liver glucose-6-phosphatase (G6P) activity, increases in liver triglycerides, and increases in SGPT activity. TCI significantly affected two of the four hepatotoxicity parameters, liver weight, and G6P activity, in a dose-related manner. Increases of liver triglycerides and increases of serum SGPT occurred only at the highest dosage levels. However, dose-related percentage increases of liver weight and of percentage inhibition of G6P activity exhibited biphasic curves (Figures 4-13 and 4-14) exactly analogous to the dose-related metabolism curve (Figure 4-8). In each case, the relationships between dose and toxic effect, and dose and metabolism were linear up through 1600 mg of TCI/kg/day, but reaching a plateau at higher doses. Furthermore, when these same hepatotoxicity data points were plotted against the amount of metabolism (i.e., total urinary metabolites) at each respective dose, linear relationships were observed throughout the entire dose range (Figures 4-13 and 4-14). These observations indicate that the nonlinearity seen in the dose-toxic effect relationships can be fully explained by the kinetics of TCI metabolism. Chemical and histopathologic examination confirmed that the dose-related toxic responses, liver weight increase, and liver G6P activity decrease were paralleled by cellular change. The increases in liver size were attributable to hypertrophy of the liver cells, since a dose-related decrease in the DNA concentration (mg/g liver) was found. Microscopic examination revealed evidence of dose-related cellular damage with cellular degeneration, swollen hepatocytes, karyorrhexis, and central lobular necrosis. Hepatic cells with two or more nuclei or enlarged nuclei containing increased amounts of chromatin were observed, suggesting that a regenerative process was ongoing.

As TCI hepatotoxicity in mice parallels TCI metabolism, then covalent binding of reactive metabolites to cellular macromolecules should also be directly proportional to the extent to which TCI is metabolized. Presently, there is less information about a possible dose-related binding of "reactive" metabolites to macromolecules that results in an incremental increase in a toxic liver response; nor have the critical target binding sites been identified. However, numerous factors, such as species, age, sex, and diet, all of which modify TCI microsomal metabolism, also markedly influence the toxic response. Drugs such as phenobarbital (Carlson, 1974) and ethanol (Cornish and Adefuin, 1966) greatly influence TCI metabolism and hepatotoxicity. Cornish et al. (1977) found that TCI-induced liver toxicity in rats, as measured by plasma SGOT, is potentiated by both ethanol and phenobarbital. Ethanol acted by competitive substrate inhibition, and also by stimulation of microsomal mixed-function oxidase system; phenobarbital potentiated toxicity by induction of mixed function oxidase system and thereby increased TCI metabolism. Allemand et al. (1978) observed a similar potentiation of toxicity in

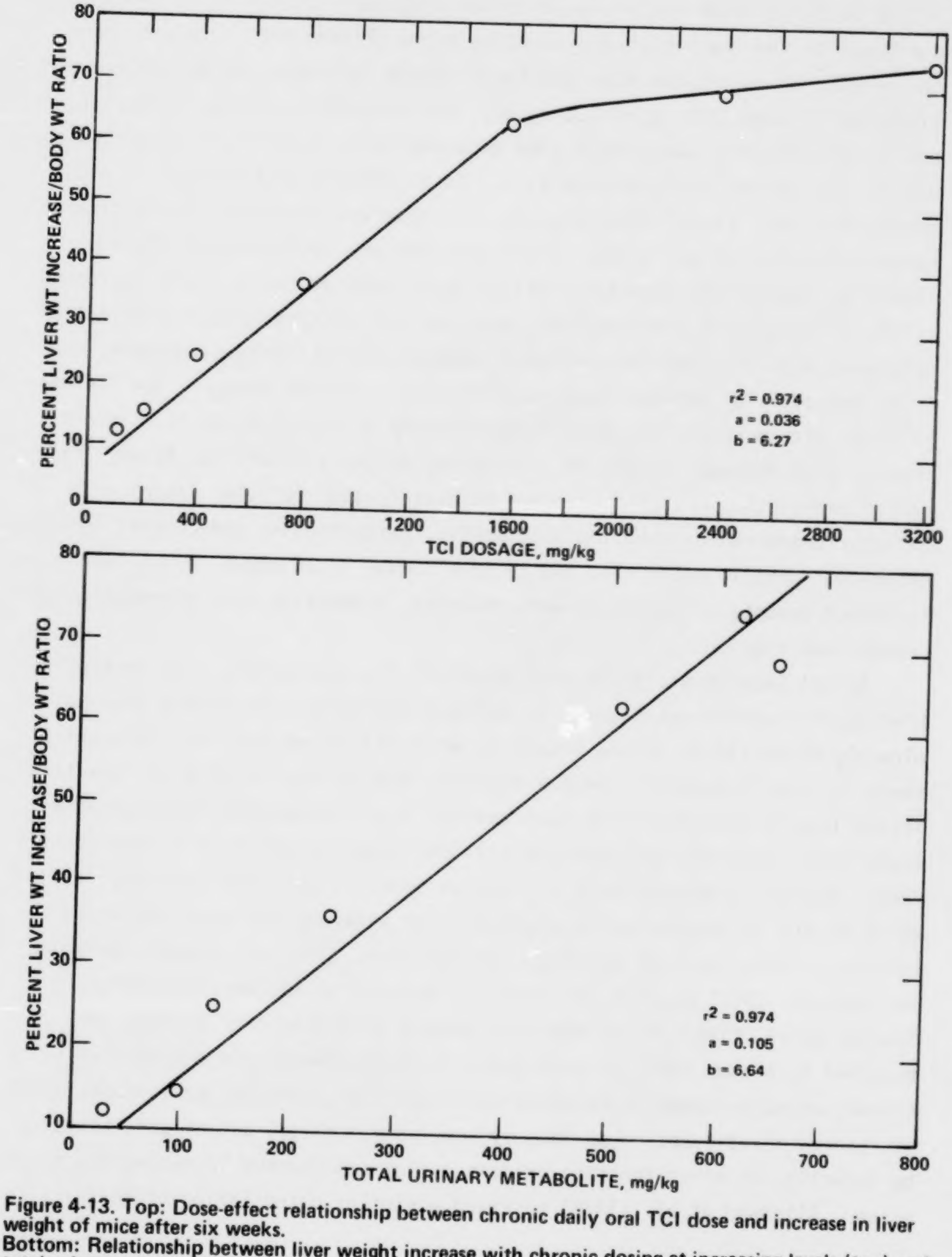


Figure 4-13. Top: Dose-effect relationship between chronic daily oral TCI dose and increase in liver weight of mice after six weeks.
Bottom: Relationship between liver weight increase with chronic dosing at increasing levels (top) and total urinary metabolite (TCA and TCE) excreted per day by mice at various dose levels.

Source: Buben and O'Flaherty (1984).

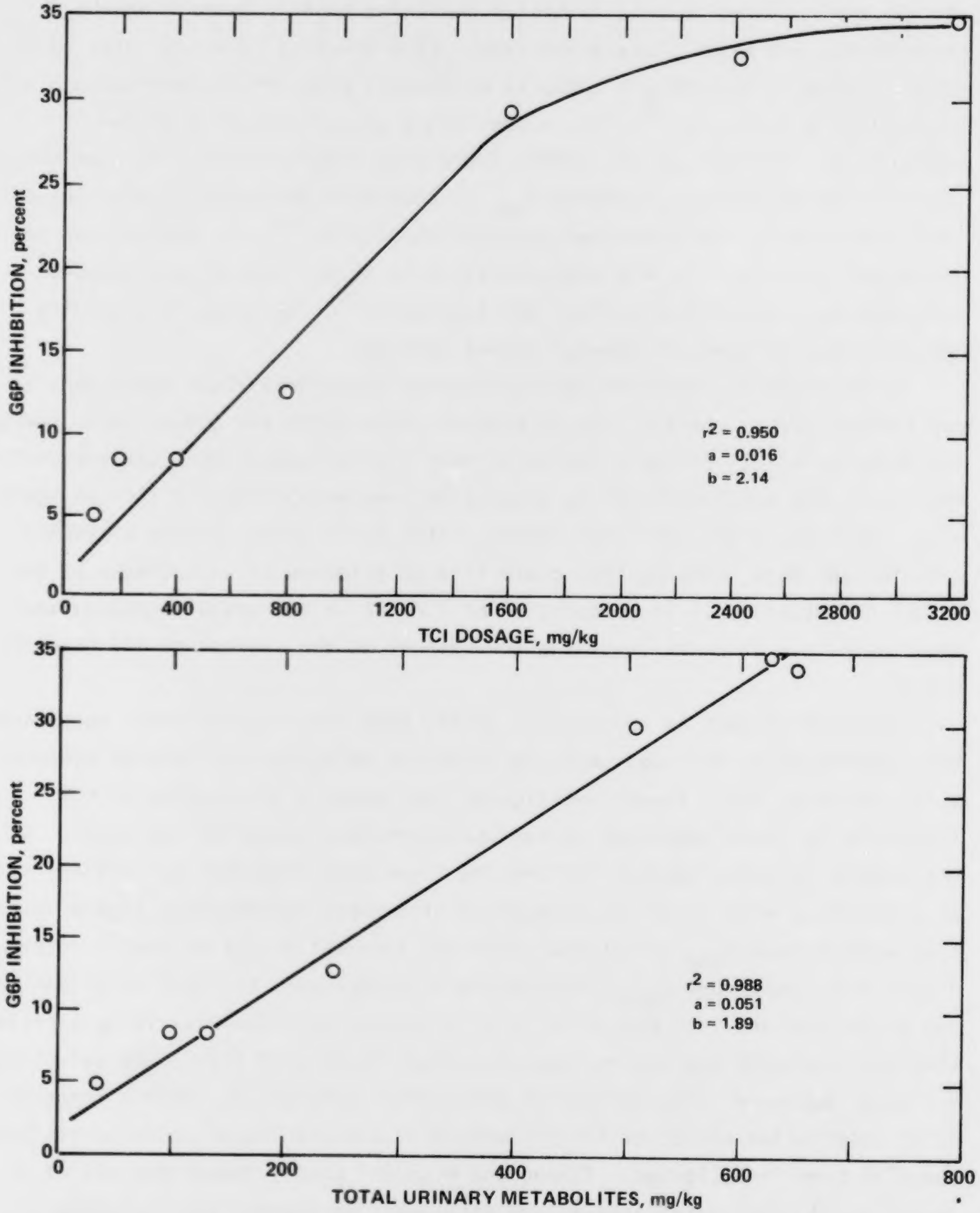


Figure 4-14. Top: Dose-effect relationship between chronic daily oral TCI dose and inhibition of liver glucose-6-phosphatase activity (G6P) of mice after six weeks.
Bottom: Relationship between inhibition of liver G6P activity with chronic dosing at increasing levels (top) and total urinary metabolite (TCA and TCE) excreted per day by mice at the various dosages.

Source: Buben and O'Flaherty (1984).

Reduced glutathione had a protection effect against TCI liver toxicity. TCI acutely administered to rats initially decreased hepatic glutathione by 61 percent, but increased glutathione levels (106 percent) 16 hours later (Moslen et al., 1977b). Glutathione added to microsomal preparations decreased binding of reactive products of ^{14}C-TCI, suggesting a glutathione conjugation of metabolites. Pessayre et al. (1979) found that single or acute TCI administration to rats decreased cytochrome P_{450} of phenobarbital-treated rats, but not untreated animals, and increased covalent binding of ^{14}C-TCI metabolites to microsomal protein. Chronic administration to normal rats simultaneously decreased P_{450} cytochrome content and increased ^{14}C-TCI metabolite binding, but also induced other microsomal enzyme systems.

Since acute TCI exposure rarely produces functional liver impairment in man (Smith, 1966), whereas chronic exposure does (Litt and Cohen, 1969; Baerg and Kimberg, 1970), Pessayre and co-workers (1979) suggest that the explanation may lie in the ability of TCI to enhance its own metabolism and its own toxicity. However, Green and Prout (1984), after daily gavage dosing of B6C3F1 mice for 180 days (1000 mg/kg), could find no evidence of an increase of the amount of metabolism. The proportion of TCA/TCE in the urine increased and thus suggests a stimulation of the TCA pathway at the expense of TCE (Section 4.2.3).

Poplawski-Tabarelli and Uehleke (1982) have investigated other mechanisms that contribute to the toxic manifestations of halogenated aliphatic hydrocarbons, including TCI. These investigators attempted a correlation of the inhibition by these compounds of various microsomal oxidative reactions. Of the several possible factors influencing these mono-oxygenase activities, such as interaction with lipid environment of microsomal cytochromes; ligand formation with reduced P_{450} cytochrome; covalent interactions of metabolic intermediates with cytochrome P_{450}; destruction of cytochromes by lipid peroxidation; and production and interaction of CO with cytochromes, the overriding correlation appeared with the simple physicochemical factors of high lipid solubility and vapor pressure. The ability of chlorinated ethylene to inhibit mitochondrial respiration and hence ATP production as a mechanism of cellular toxicity has also been investigated. Takano and Miyazaki (1982) found that all of a series of 14 chlorinated ethanes and ethylenes, including TCI, inhibited oxygen consumption of isolated rat mitochondria; their inhibitory potency increased in the order of the number of contained chlorines.

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5. TOXICOLOGICAL EFFECTS IN MAN AND EXPERIMENTAL ANIMALS

5.1 INTRODUCTION

Trichloroethylene (TCI) has been an important industrial chemical since its commercial application in 1906. It has been the focus of wide research interest and the subject of numerous case reports. The available literature concerning the potential effects of TCI is reviewed in this chapter.

5.2 NEURAL AND BEHAVIORAL EFFECTS

Ostlera (1953), in a historical discussion of TCI, cited descriptions of its anesthetic, analgesic, and neurotoxic effects from reports as early as 1911. The symptoms described are remarkably similar to those found in more modern literature. In 1931, Stuber reviewed a total of 283 cases of presumable overexposure to TCI, including 26 fatalities. Principal findings indicated involved neural and behavioral effects. No cases of injury to the liver were reported. The above findings were further supported by a study of 384 cases of industrial gassing poisonings by TCI and other halocarbons from 1961 to 1980 (McCarthy and Jones, 1983). TCI exposure was attributed to 288 reports. The majority of symptoms were attributed to effects on the central nervous system (CNS). However, gastrointestinal and respiratory symptoms also were quite common. The observation that TCI caused paralysis of the trigeminal nerve led to its early use in the treatment of trigeminal neuralgia. Boulton and Sweet (1960) suggested that trigeminal palsies that occurred in patients under TCI anesthesia may have resulted actually from inhalation of dichloroacetylene or phosgene, formed when exhaled TCI was passed through soda lime to remove carbon dioxide (CO_2). The formation of these impurities and their subsequent toxicity resulted in a decline in the use of TCI as an anesthetic.

5.2.1 Human Studies

5.2.1.1 Short-Term Exposures.

5.2.1.1.1 Case reports. Numerous case histories have described the effects of short-term exposure to high levels of TCI (Feldman et al., 1970; Longley and Jones, 1963; Harenko, 1967; Masoero and Lavarmo, 1955; Vallee and Leclercq, 1935; Kleinfeld and Tabershaw, 1954; Tomasini and Sartorelli, 1971; Mitchell and Parsons-Smith, 1969; Maloof, 1949; Steinberg, 1981; Lawrence and Partyka, 1981). The earlier studies reported on the toxicity of TCI following anesthesia, in particular the occurrence of trigeminal palsies (Humphrey and McClelland, 1944). Effects reported included dizziness, headache, nausea, confusion, and, at very high levels, unconsciousness. Facial numbness and blurred vision also were reported occasionally. Psychotic symptoms resulted from ingestion of TCI and alcohol (Harenko, 1967).

5.2.1.1.2 Experimental exposures. Stewart et al. (1970) exposed six humans to 200 ppm (1076 mg/m^3) TCI for 7 hours. The subjects reported rapid adaptation to the odor of TCI. Some complaints of dryness of the throat and mild eye irritation occurred after the first 30 minutes of exposure.

Experimental exposures to 95 ppm (511 mg/m^3) (Konietzko et al., 1975) or up to 300 ppm (1644 mg/m^3) for 2.5 hours (Ettema et al., 1975) had no effect on such behavioral task performances as simple or choice reaction time, hand steadiness, tapping tests, or pursuit tracking. While Ettema et al. (1975) reported slight effects, repeated significance tests never produced a p value below 0.10 and effects were not dose-related.

Vernon and Ferguson (1969) exposed eight males repeatedly to 0, 100, 300, or 1000 ppm (0, 538, 1644, or 5380 mg/m^3) at 2-hr intervals, with 3 days separating sequential exposures. Statistical tests showed significant decrements in the performance of groove-type hand steadiness, depth perception, and pegboard tests at 1000 ppm (5380 mg/m^3) only. It is noteworthy that plots of trends of the scores demonstrated nonsignificant increases in errors at levels lower than 1000 ppm (5380 mg/m^3). Trend tests or studies with a larger group of subjects perhaps would have been more informative. No effects were noted on Muller-Lyer illusions, flicker fusion, and code substitution tests.

Ferguson and Vernon (1970) repeatedly exposed individuals to 0, 300, or 1000 ppm (0, 1644, or 5380 mg/m^3) TCI for 2 hours. In one group of eight males, individuals were given also either meprobamate (800 mg) or an antihistamine, thonzylamine hydrochloride (50 mg). In a second group of six exposed to TCI as above, an alcoholic drink sufficient to produce a blood alcohol level of 20 to 30 mg/100 ml blood (2 mg/kg) was also given. By itself, TCI exposure produced decrements in depth perception, hand steadiness, and pegboard performance that were statistically significant only at 1000 ppm (5380 mg/m^3). The two drugs in addition to TCI exposure produced no effects beyond that induced by TCI exposure alone. Alcohol, however, substantially augmented the effects of TCI such that effects were significant at 300 ppm (1644 mg/m^3). Flicker fusion and Muller-Lyer perception were unaffected by any of the conditions.

Windemuller and Ettema (1978) exposed four groups of six males each for 2.5 hours to either (a) room air only, (b) 200 ppm TCI, (c) 1 ml/kg alcohol in a mixed drink, or (d) both alcohol and TCI. Performance was measured in a pursuit rotor task or by a binary choice reaction time test. Heart rate, sinus arrhythmia, and respiration rate also were measured and interpreted as correlates of "mental load." The only variable affected was sinus arrhythmia, which was significantly reduced only by the combination of 200 ppm (1076 mg/m^3) TCI and alcohol. According to the authors, behavioral performance was at too high a level to show any decrement due to the experimental conditions. A separate group of 15 subjects was studied in a design in which all participated under all conditions. The same results obtained.

In a demonstration project, Stopps and McLaughlin (1967) showed that TCI exposure involving one individual caused slight effects. During a 1.5-hour exposure, TCI was administered sequentially in first an ascending and then descending order (100, 200, 300, or 500 ppm; 538, 1076, 1644, or 2690 mg/m^3). Of a battery of tests, the Necker cube reversal illusion was considerably affected. Because only one individual was exposed, such findings are difficult to interpret. Kylin et al. (1967) purported to show that optokinetic nystagmus in 12 humans was affected by 1000 ppm (5380 mg/m^3) TCI for 2 hours. However, data were graphically displayed only and thus trends are difficult to see.

5.2.1.2 Long-Term Exposure

5.2.1.2.1 Case reports. Evaluation of case reports indicates that symptoms involved in short-term exposure situations also are present in long-term exposures but in more extreme and persistent forms (Harenko, 1967; James, 1962; Kleinfeld and Tabershaw, 1954; Mitchell, 1969; and Steinberg, 1981). Dizziness, nausea, and headache persist even after cessation of daily exposure. Longer exposures may produce ataxia, decreased appetite, sleep disturbances, and perhaps even psychotic episodes. Trigeminal neuropathy was reported in two cases (Mitchell, 1969; Feldman et al., 1970). In some instances recovery was reported, but in others, permanent damage seems to have occurred. Data are lacking so that it is not possible to characterize the parameters of exposure beyond which irreversible damage occurs. In some instances, death occurred (Kleinfeld and Tabershaw, 1954; James, 1963). In four of six cases, death occurred from 1 to 17 hours after cessation of exposure. In a case of

TCI ingestion, death occurred several days after the incident. Kleinfeld and Tabershaw (1954) conjectured that death was due to ventricular fibrillation. It is possible that in cases where repeated TCI exposures were not sufficient to produce anesthesia, a day-to-day accumulation took place in body tissues (see Chapter 4).

5.2.1.2.2 Workplace exposure. Formal symptom surveys of workers exposed to various levels of TCI on a daily basis revealed that many symptoms of short-term exposures became chronic complaints. Grandjean et al. (1955) studied 73 workers, Bardodej and Vyskocic (1956) studied 75, and Lilis et al. (1969) studied 70 workers. Complaints included vertigo, fatigue, headache, and nausea. As in case reports, tremors, ataxia, and impaired vision also were found. Reported alcohol intolerance in TCI-exposed workers could be due to day-to-day accumulation of TCI in tissue (see Chapter 4) and the interaction of alcohol with TCI. Bardodej and Vyskocic (1956) also reported frequent cases of addictive behavior in which insomnia occurred unless TCI was sniffed. In the study of Grandjean et al. (1955), complaint frequency was higher in the 85 ppm (457 mg/m^3) exposure group than in the 14 and 34 ppm (75 and 183 mg/m^3) exposure categories. Grandjean et al. (1955) also administered tests for short-term memory and for measuring understanding of instructions along with psychiatric interviews to 73 workers exposed to average levels of 14, 34, and 85 ppm (75, 183, and 457 mg/m^3), as part of the study. In the 85 ppm (457 mg/m^3) group there were higher frequencies of short-term memory loss, fewer word associations, increased perseverance, and increased rates of misunderstanding.

Workers have also been formally tested for trigeminal nerve function. Barret et al. (1982) studied 11 unexposed and 20 TCI-exposed (undocumented levels) workers. Compared to the unexposed group, 8 of the 20 exposed individuals had elevated thresholds for trigeminal nerve evoked potentials. Triebig et al. (1982) studied trigeminal motor and sensory nerve conduction velocity in 26 individuals occupationally exposed to an average of 40 ppm (215 mg/m^3) TCI and in 24 workers having no TCI exposure. No differences between groups were found, but variances were large and exposure levels small.

Konietzko et al. (1974) found EEG changes in workers exposed to TCI. The EEG for six workers was collected during work on TCI-exposed days and on nonexposure days. During exposure days (50 to 100 ppm TCI), there were more bursts of alpha waves and the alpha waves had higher amplitude than on unexposed days. No clinically abnormal EEG observations occurred during unexposed work or in a clinical setting. Increased alpha activity frequently occurs in individuals who are in a relaxed state.

5.2.1.2.3 Experimental exposure. Stewart et al. (1970) exposed six individuals to 200 ppm (1076 mg/m^3) TCI for 7 hr/day, for 5 sequential days. Subjective reports were collected in addition to blood and physiological measures. Odor perception decreased over the course of repeated exposures as did complaints of eye irritation, dryness of throat, and other mild symptoms. By the fourth and fifth days, however, fatigue and sleepiness during exposure were reported by five of the six individuals.

5.2.1.2.4 Summary of human neural and behavioral effects. The effects of short-term TCI exposure range from mild eye irritation, nausea, vertigo, headache, confusion, unconsciousness, and death, depending upon exposure concentration. Mild irritation occurs at levels near 200 ppm (1076 mg/m^3). Hand steadiness, coordination, and possibly depth perception are affected at 1000 ppm (5380 mg/m^3) and perhaps below (see Section 5.2.1). If combined with alcohol ingestion, TCI can produce these effects at levels of 200 to 300 ppm (1076 to 1614 mg/m^3).

Extended exposure can increase the duration and intensity of nausea, vertigo, and headache, but eye irritation and olfactory sensation are reduced. Confusion, reduced cognitive performance, impaired short-term memory, sleep disturbances, loss of appetite, addiction, alcohol intolerance, tremors, ataxia, and trigeminal neuropathy also are reported. The threshold for such complaints is difficult to estimate since such data are gathered from workplace surveys with all of the attendant problems in quantification and control. It appears that effects, however, are absent below 85 to 100 ppm (457 to 538 mg/m^3).

5.2.2 Laboratory Animals

5.2.2.1 Short-Term Animal Exposures. Exposure to 30,000 ppm (161,000 mg/m^3) TCI for about 20 minutes was lethal to 15 dogs (Baker, 1958). After about 5 minutes, dogs began to salivate heavily and to become ataxic. Between 5 and 10 minutes, they exhibited foreleg tonicity and became semicomatose. During the last 10 minutes, dogs were unconscious and frequently exhibited convulsive (particularly hindlimb) movements. Not surprisingly, no CNS pathology was found during autopsy.

Grandjean (1963) exposed 29 rats to 400 ppm (2152 mg/m^3) TCI and a group of 11 to 800 ppm (4304 mg/m^3) TCI, for 6 hours. Immediately upon termination of exposure, rats were tested in a swimming escape task. Rats were tested with and without a 27 gram weight attached to their tails. In each test, rats were given ten sequential trials. At 400 ppm (2152 mg/m^3) no effects of TCI were observed without the added load and only slight slowing effects were seen with the added load. At 800 ppm (4304 mg/m^3) effects were slight and inconsistent without the added load; definite and consistent slowing of swimming time occurred with the added load. During the sequential trials, animals exposed to 800 ppm (4304 mg/m^3) TCI under load showed the greatest effects on the eighth, ninth, and especially the tenth trial. The author interpreted these data to mean that TCI exposure produced greater fatigue since the effects were dependent upon load and time.

In the above report, Grandjean (1963) also presented data on jiggle cage activity levels from 12 rats per group exposed to 0, 400, 800, or 1200 ppm (0, 2152, 4304, or 6456 mg/m^3) TCI for 5 hours. Activity was measured during exposure and again for a period from 20 to 80 minutes after exposure. Activity levels were significantly decreased during and after exposure to 1200 ppm (6456 mg/m^3). Means of the 400 and 800 ppm (2152 and 4304 mg/m^3) groups also were lower but not significantly so.

Grandjean (1960) exposed rats for 8 hours to either 200, 600, or 1600 ppm (1076, 3228, or 8608 mg/m^3) TCI which were then subsequently tested for exploratory behavior in a maze. Exploratory behavior was reported to have decreased at 200 and 1600 ppm, but increased at 600 ppm (3228 mg/m^3). Since no actual data were presented, the information is difficult to interpret.

Baetjer et al. (1970) used hypothalamic electrical stimulation to reinforce lever pressing behavior in rats. Groups ranging in size from 14 to 36 were exposed to either 0, 2500, or 3500 ppm (0, 13, 450, or 18,830 mg/m^3) TCI for 30 minutes on three sequential days. At each exposure level, one group was placed on water deprivation for 3 days while the other group was not. Thus, there were six groups of rats. TCI at both levels reduced the lever pressing rate in a dose-dependent fashion. Especially at 2,500 ppm (13,450 mg/m^3), response rate reduction was less noticeable on day 2 than on day 1 and on day 3 than on day 2. Thus, it appears that short-term adaptation can occur. Water-deprived rats were affected less and had delayed effects in comparison to normals. The authors speculate that in brain cells of dehydrated rats, either TCI levels accumulated more slowly or the brain cells were more resistant to TCI effects. Another possible interpretation is that the interoceptive stimuli from water deprivation plus the stimuli associated with TCI exposure simply increased the activity and, as a result, the lever pressing rate, with respect to rats that were subjected to only one of the above. Hence, dehydration may have been unimportant except as another source of stimulation.

5.2.2.2 Repeated Exposures (4 days to 5 weeks). Savolainen et al. (1977) recorded open-field activity in rats after the last of 4 days of exposure to either 0 or 200 ppm (0 or 1076 mg/m^3) TCI for 6 hr/day. During the first hour after exposure, the activity level was higher in the exposed group, but by 17 hours postexposure the effect was no longer present.

Silverman and Williams (1975) exposed independent groups of 16 rats to either 100, 200, 500, or 1000 ppm (538, 1076, 2690, or 5380 mg/m^3) TCI for 6 hr/day, 5 days/week, for 5 weeks. For each of the abovementioned four groups, a separate contemporaneous group of 16 unexposed controls was studied. The activity of pairs of male rats was studied in their home cages for 5 minutes after termination of exposure on days 1, 3, 10, 17, and 24. Where significant TCI effects occurred, the effect was always a reduction of activity. At 1000 ppm (5380 mg/m^3), the effect was evident on the first day. Other levels of exposure showed a gradual decline in activity with the decline reaching a criterion of significance in a dose-dependent fashion. The 100 ppm (538 mg/m^3) group was not affected significantly at the end of 5 weeks and was further exposed for a total of 8 weeks. This group was eventually affected in a significant manner.

Goldberg et al. (1964) exposed groups of rats to 200, 560, 1568, and 4380 ppm (1076, 3013, 8436, and 23,564 mg/m^3) TCI for 4 hr/day, 5 days/week, for 2 weeks. Signaled electric shock escape-avoidance behavior was studied in previously trained rats. The highest exposure level produced a significantly greater rate of response inhibition than did any of the lower concentrations. Concentrations from 200 to 1568 ppm (1076 to 8436 mg/m^3) did not differ. Another group of rats was trained on the same task during exposure. No significant differences were found although asymptotic performance was reduced in a dose-related manner above 200 ppm (1076 mg/m^3). The 200 ppm group seemed to reach the asymptotic level faster than controls. It must be emphasized that none of the acquisition curves were significantly different from each other and the reported effects should, therefore, be viewed with caution.

Battig et al. (1960) exposed four groups of six rats each to either 0 or 600 ppm (0 or 3228 mg/m^3) TCI for 3 to 4 hr/day. The daily exposure plan was complex, with each group receiving TCI at least once during acquisition and extinction of a shuttle box escape-avoidance task. In no case was acquisition affected by TCI exposure, but reaction times were increased and extinction was slowed.

Three rats were trained in positively reinforced, rope-climbing behavior under the control of a positive discriminative stimulus (Grandjean, 1960). They were then tested immediately after exposure to either 0, 200, or 800 ppm (0, 1076, or 4304 mg/m^3) TCI. Apparently there were 7 sequential days at 200 ppm (1076 mg/m^3) for 3 hr/day, followed by 5 days of 800 ppm (4304 mg/m^3) for 3 hours. Controls were interspersed. It is not clear when the data presented were collected; they are probably a summary over all days. There was no effect on latency or failures to respond, but the number of responses in the absence of a discriminitive stimulus was higher in the TCI-exposed groups. There was no difference between the 200 and 800 ppm (1076 and 4304 mg/m^3) groups. Little can be concluded from only three rats.

Goldberg et al. (1964) reported data on the performance of electric shock escape-avoidance responses in 10 rats exposed to 125 ppm (672 mg/m^3) TCI for 4 hr/day, 5 days/week, for a total of 14 days. Rats were tested during the last hour of exposure. At the onset of exposure, rats immediately began to receive more electric shocks (avoidance response failures) and continued to perform more poorly than on pre-exposure days throughout the 14 days. Some evidence of adaptation to TCI was observed. After the 14-day exposure period, rats were tested for 7 more unexposed days during which performance returned to and below pre-exposure levels. The evidence for adaptation is poor since (a) no contemporaneous controls were studied and (b) the "adaptation" trend of improvement continued for the 7 postexposure days.

5.2.2.3 Long-Term Exposures. Battig and Grandjean (1963) exposed groups of 10 rats to either 0 or 400 ppm (0 or 2152 mg/m^3) TCI for 8 hr/day, 5 days/week, for 44 weeks. Several behaviors were tested either immediately after daily exposure or 16 hours after exposure. TCI increased times in a swimming escape-avoidance test. The magnitude of the effect became greater as the number of weeks of exposure increased. When tested 16 hours after exposure, no effect was seen on the swimming test. Acquisition of shuttle box avoidance behavior was not affected either immediately after exposure or 16 hours later. Hebb maze performance was not affected when tested 16 hours after exposure. Tests of exploratory behavior which were conducted immediately after exposure during the last 2 weeks of the study showed an increased level of activity. This may be due to the lack of adaptation to novel surroundings (Daschiell maze).

Kjellstrand et al. (1980) studied the effects of continuous 320 ppm (1721 mg/m^3) exposure for 9 months on radial-arm maze performance in 24 gerbils. An unexposed control group of 24 also was tested. Gerbils were removed from exposure chambers for the test. An eight-arm maze test was started on day 59 and terminated on day 87. Performance was nearly perfect in both groups. At day 237, a 16-arm maze test was begun with 16 gerbils per group. Again, no significant differences were observed even though performance was not as good as in the eight-arm maze. Eleven days after the termination of the 9-month exposure, 32 gerbils were split into four groups: two groups of 8 previously exposed to 9 months of TCI and two groups of 8 previously unexposed. All four groups were exposed on alternate days to 2,300 ppm 1,1,1-trichloroethane for 6 hours and then tested in the 16-arm maze. Those gerbils that had previously been exposed to TCI showed worse performance after 1,1,1-trichloroethane exposure than after air exposure. Similar but somewhat less dramatic results were obtained from tests 75 days after TCI exposure. The authors interpreted these findings to mean that permanent damage to the CNS resulted during the 9 months of exposure to TCI. This interpretation is difficult to support since exposure to 1,1,1-trichloroethane improved performance in gerbils previously exposed to TCI. It is more likely that the findings can be explained in terms of state dependent learning.

Baker (1958) exposed dogs repeatedly to concentrations of TCI ranging from 500 to 3000 ppm (2690 to 16,140 mg/m^3) for 2 to 8 hours. He reported anecdotally that animals exhibited such chronic symptoms as ataxia, excessive salivation, head tremor, teeth gnashing, and extension of extremities. Upon repeated exposure, dogs progressively became unconscious more quickly. Postmortem inspection of brain tissue in three dogs that had survived between 60 and 162 hours of TCI (unspecified concentration) revealed selective destruction of cells in the Purkinje layer of the cerebellum and, to a lesser extent, unselective cellular damage to the cerebral cortex. Changes to Purkinje cells in rabbits injected intramuscularly with a total of 53 g of TCI were reported by Bartonicek and Brun (1970).

Continuous exposure of gerbils to 0, 60, or 320 ppm (0, 323, or 1722 mg/m^3) TCI for 3 months was followed by a recovery period of 4 months (Haglid et al., 1981). Gerbils were sacrificed (6 to 7 per group) and the percentage of S100 protein and DNA in various brain regions was determined. Although the authors purport to find a consistent change in S100 protein percentages, inspection of the data reveals changes which are not dose-related and are inconsistent across areas. DNA seems to be increased in the cerebellum, sensory cortex, and motor cortex, but not in brainstem or hippocampus. However, if one makes Bonferroni corrections to the significance tests, no significant increases remain. This report should be viewed as presenting pilot data only.

5.2.2.4 Summary of Neural and Behavioral Effects in Laboratory Animals. Effects of TCI upon behavior have been demonstrated at exposures as low as 400 ppm (2154 mg/m^3)for 6 hours or as low as 200 ppm (1076 mg/m^3) for four, 6-hr days. If exposure is extended to 6 hr/day for up to 8 weeks, 100 ppm (538 mg/m^3) will produce behavioral effects. These effects include increased fatigue, decreased activity level, and slowed extinction of learned behaviors.

These behavioral changes might well be regarded as analogous to the effects of TCI on humans. Fatigue is a common complaint at low exposure levels. Activity level reduction is another possible analog of fatigue. Slowed extinction of a learned response might be related to short-term memory loss. In general, the laboratory animal as an analog of human behavior is speculative.

At very high repeated exposure levels, TCI apparently selectively destroys the Purkinje cell layer in the cerebellum. Data regarding the neuropathological effects of lower level exposures for long periods are inconclusive or absent. Neuropathological investigations could provide a model of TCI-induced ataxia.

5.3 EFFECTS ON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS

5.3.1 Cardiovascular Effects

The use of TCI as an anesthetic has been associated with cardiac arrhythmias, including bradycardia, atrial and ventricular premature contractions, and ventricular extrasystoles. The dose-response relationships for these effects, however, has not been established in man or experimental animals. High concentrations of TCI have been reported to sensitize the myocardium to circulatory catecholamines, resulting in cardiac arrhythmias including ventricular fibrillation or even cardiac arrest (Dhuner et al., 1951, 1957; Reinhardt et al., 1973). Metabolites of TCI, such as chloral hydrate, may also be associated with arrhythmias involving adrenergic stimulation (DiGiovanni, 1969). Lilis et al. (1969) observed in their study that 60 percent of the workers exposed to inhaled TCI exhibited an increase in cardiac output, an effect attributed to increases of epinephrine release and circulating plasma levels. These direct cardiac effects of TCI, with sensitization to catecholamines, have been observed after accidental ingestion and have, in some cases, proved fatal (Defalque, 1961).

Several case histories have been reported involving fatalities following acute exposure to large concentrations of TCI. The deaths in these cases were attributed to either cardiac arrest (James, 1963) or ventricular fibrillation (Kleinfield and Tabershaw, 1954). Bell (1951), Bardodej and Vyskocil (1956), Ogata et al. (1971), Andersson (1957), and others have noted that exposure to TCI may either speed up or slow down the heart rate, depending on the degree of exposure. The most direct evidence that TCI causes ventricular extrasystoles, fibrillation, and cardiac arrest comes from changes that are observed in the electrocardiograms (EKG) of subjects accidentally exposed to high concentrations of TCI (Kledecki and Bura, 1963; Mroczek and Fedyk, 1971; Yacoub et al., 1973). Bernstine (1954) recorded ventricular fibrillation confirmed by EKG in a worker who sustained cardiac arrest after inhalation of TCI in analgesic concentrations. Barnes and Ives (1944) found that 33 of 40 normal patients in deep anesthetic planes showed signs of ventricular extrasystoles and multifocal ventricular tachycardia.

Few systematic animal studies have been done on the effects of TCI on the cardiovascular system. Mazza and Brancaccio (1967) studied the effects on rabbits of chronic exposures to 2790 ppm (15,010 mg/m^3) TCI, 4 hr/day, 6 days/week, for 45 days. They found that severe blood dyscrasia was produced in these animals. The characteristics of the hemochromocytometric tests and the description of the bone marrow led the authors to conclude that chronic intoxication with TCI has a direct effect on the bone marrow, which causes myelotoxic anemia. Mikiskova and Mikiska (1966) studied electrophysiological responses of guinea pigs injected i.p. with either with 6.7 mM/kg TCI or with 2.2 mM/kg trichloroethanol. They recorded EKG's and EEG's and found that trichloroethanol was at least three times as effective as TCI in inducing effects on the nervous system and in slowing the heart, and suggested that TCI effects were due, in part, to its metabolite, trichloroethanol.

White and Carlson (1979) demonstrated that epinephrine-induced arrhythmias in rabbits exposed to TCI could be decreased by phenobarbital treatment. This increases metabolism of TCI. This would support the view that TCI, and not its metabolites, is responsible for arrhythmias. Caffeine has been shown to potentiate the arrhythmogenicity of TCI in rabbits upon challenge with exogenously administered epinephrine (White and Carlson, 1982). Rabbits were treated with a vehicle control (1 mg/kg, i.p.) or caffeine (10 mg/kg, i.p. 30 minutes prior to exposure) and exposed for 1 hour to 6000 ppm (32,280 mg/m^3) TCI under dynamic airflow conditions. Epinephrine was infused until arrhythmias occurred after 7.5, 15, 30, 45 and 60 minutes of exposure and 15 and 30 minutes post-exposure. The authors reported a pronounced increase in the incidence of epinephrine-induced arrhythmias in TCI-exposed rabbits when treated with caffeine and challenged with doses of epinephrine as low as 0.5 µg/kg.

White and Carlson (1981) also investigated the effect of ethanol on epinephrine-induced cardiac arrhythmias in rabbits exposed to 6000 ppm (32,280 mg/m^3) TCI. Rabbits were treated with saline (1 ml/kg) or ethanol (1 g/kg, i.v. or p.o.) 30 minutes prior to exposure to 6000 ppm TCI for 1 hour. Epinephrine was infused as described above. Rabbits treated with ethanol developed epinephrine-induced cardiac arrhythmias sooner and at lower doses of epinephrine than control rabbits. More arrhythmias developed in rabbits treated with ethanol i.v. than p.o. Ethanol administration decreased the conversion of TCI to trichloroethanol by 50 percent, and trichloroacetic acid production was completely blocked by ethanol treatment.

Most recently, Carlson and White (1984) evaluated the effect of benzo(a)-pyrene on the production of arrhythmias in epinephrine-induced, TCI-exposed rabbits. Benzo(a)pyrene increased TCI metabolism but also led to increased arrhythmias. Thus, it would appear that benzo(a)pyrene has intrinsic arrhythmogenic action. Rabbits were exposed to 8100 ppm (43,578 mg/m^3) TCI 48 and 72 hours subsequent to administration of 40 mg benzo(a)pyrene/kg.

Fossa et al. (1982) demonstrated that disulfiram exhibits a cardioprotective activity not related to its effects on TCI metabolism in rabbits. Disulfiram (1.35 mmoles/kg, p.o.) was administered 24 and 6 hours prior to a 1-hr exposure to 6000 ppm (32,280 mg/m^3) TCI. Disulfiram or its metabolites, CS_2 and diethyldethiocarbamate (1.35 mmoles/kg, i.p.), were administered at similar time intervals also to rabbits exposed for 1 hour to 9000 ppm (48,420 mg/m^3) TCI. Blood levels of TCI and its metabolites, trichloroethanol and trichloroacetic acid, were measured at periods ranging from 7.5 minutes of exposure to 30 minutes post-exposure. The metabolism of TCI was inhibited in all treatment groups. When challenged with 0.5 to 3.0 µg/kg epinephrine, disulfiram prevented the epinephrine-induced arrhythmias resulting from exposure to 6000 ppm

($32,280 mg/m^3$) TCI. All treatment groups in the first 30 minutes of exposure to 9000 ppm ($48,420 mg/m^3$) TCI exhibited an increased percentage of animals responding with arrhythmias compared to controls. Thirty to sixty minutes of exposure resulted in a significant decrease in the percentage of animals responding with arrhythmias in the disulfiram- and diethyldithiocarbamate-treated groups.

5.3.2 Respiratory Effects

In anesthetic concentrations, TCI causes little or no irritation to the respiratory tract (Dobkin and Byles, 1963). A striking effect is observed, however, on the rate and depth of respiration. TCI characteristically causes increased respiratory rate (tachypnea) but decreased alveolar ventilatory amplitude, which is associated with decreased blood oxygen tension and increased carbon dioxide tension (Dobkin and Byles, 1963). These effects were noted in early animal experiments (Lehmann, 1911) and in early reports on human patients under TCI anesthesia (Striker et al., 1935). The incidence of tachypnea increases as the concentration of TCI increases (Atkinson, 1960).

To investigate the mechanism responsible for the increase in respiratory rate, Whitteridge and Bülbring (1944) studied the effects of TCI on pulmonary afferent nerve endings in cats. They found that 0.5 to 3.5 percent (5,000 to 35,000 ppm; 26,900 to $188,300 mg/m^3$) TCI produced a dose-dependent increase in the frequency of vagal discharge and an increased sensitivity of the stretch endings. Coleridge et al. (1968) observed similar results in isolated pulmonary receptor endings.

Coleridge et al. (1968) attempted to correlate injury to rat pulmonary alveolar parenchyma with biochemical alterations following repeated inhalation of TCI. Mild changes in morphology were observed, including condensed mitochondria and vacuolation of the cells. Pulmonary surfactant secretion was significantly decreased. The authors suggested that modification of surfactant secretion was a consequence of nonspecific injury following exposure of a susceptible cell population to TCI.

Lewis et al. (1984) attempted to determine if TCI alone (without previous induction) can affect the activity of pulmonary mixed-function oxidase in mice and if inhalation of this compound can injure the lungs directly. Animals were exposed to 10,000 ppm TCI ($53,800 mg/m^3$) for 5 days, 1 or 4 hr/day. Pathologic changes such as thrombus formation and vacuolization of bronchiolar epithelial cells were also noted in the animals with reduced microsomal NADPH cytochrome c reductase. The authors concluded that the decrease in pulmonary enzyme activity may reflect direct damage to the lungs.

5.4 HEPATIC AND RENAL TOXICITY

5.4.1 Human Studies

The similarity between TCI and chloroform and other related halocarbons known to cause liver damage suggests that TCI might possess hepatotoxic activity. Stuber (1931) reviewed 284 cases of industrial poisoning due to TCI and found no evidence of liver damage. Brittain (1948) found no evidence of liver or kidney damage in 250 neurosurgery patients who underwent prolonged TCI anesthesia. However, fatal acute hepatic failure has been observed following the use of TCI as an anesthetic. Such failure has generally been in patients with other complications such as malnutrition, toxemias and burns, or patients who had received transfusions (Ayre, 1945; Berleb, 1953; Dodds, 1945; Elam, 1949; Heizer, 1951; and Werch et al., 1955).

Secchi et al. (1968) reported acute liver disease in three of seven cases of poisoning following ingestion of TCI. They suggested, however, that the results could be attributed to contamination with 1,2-dichloropropane and 1,2-dichloroethane since no liver damage was associated with ingestion of pure TCI. Cotter (1960) described 10 patients acutely exposed to TCI in a confined space. Four patients showed hyperglobulinemia and six exhibited hypercalcemia. No other liver tests were abnormal and, in all but one person, the clinical tests returned to normal within two months of exposure. Lachnit and Brichta (1958) reported that of 22 workers occupationally exposed to TCI, only three had abnormal reactions on two liver function tests (sulfobromophthalein clearance tests and colloid stability). Albahary et al. (1959) conducted liver function tests--measuring serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)--on workers regularly exposed to TCI. No evidence of liver disorders was found. Guyotjeannin and Van Steenkiste (1958) found some abnormalities in cephalic flocculation, total lipids, and plasma unsaturated fatty acids, and an increase of plasma gamma globulins in 18 workers regularly exposed to TCI. Tolot et al. (1964) found no evidence of liver injury, even though workers were routinely exposed to TCI at concentrations higher than the allowable tolerance limits, that is, 0.9 to 3 mg of TCI per liter of air (167 to 558 ppm). Joron et al. (1955) reported massive liver necrosis (particularly the left lobe) followed by death of a chemist exposed intermittently to TCI over a 1.5-year period. Subsequent analyses of room air indicated TCI concentrations as high as 300 ppm (1614 mg/m^3).

Phoon et al. (1984) reported recently on five individuals exposed to TCI occupationally who had liver damage in conjunction with severe erythema (Stevens-Johnson syndrome). It is unlikely that TCI was a causative factor in producing the liver injury since other workers were unaffected and since the exposure levels were estimated to be between 9 and 169 ppm (50 and 912 mg/m^3).

In the case reports of occupational exposure to TCI described by Suciu and Olinici (1983), factors other than TCI probably were more causally related to the hepatorenal disturbances noted.

From the above reports, it is apparent that observations of liver or renal dysfunction in workers exposed to TCI have been infrequent, and relatively little surveillance has been conducted to ascertain latent effects. Thus, it is unlikely that the potential magnitude of any kidney or liver abnormalities in humans exposed for long periods to TCI is fully known. Cases of severe liver and kidney damage resulting from ingestion of TCI may provide some evidence for latent toxicity. However, it is difficult to predict whether the same effects would be observed if ingestion of TCI was spread over a period of time similar to that found in inhalation exposure.

TCI has also been reported to have some abuse potential. Cases of deliberate and repetitive inhalation have been reported by James (1963) and by Ikeda and Imamura (1973). Baerg and Kimberg (1970) documented centrilobular hepatic necrosis and acute hepatic and renal damage following repeated sniffing of cleaning fluid containing TCI and petroleum distillates by teenagers. However, as the vapor concentration was high and inhalation was only for a short period, the effect cannot be directly extrapolated to inhalation of the same total dose over a long period of time, as in occupational exposure.

5.4.2 Animal Studies

Hepatotoxic potential has been evaluated in a number of animal species under various exposure conditions and schedules. Findings are tabulated in Table 5-1.

5.4.2.1 Inhalation Studies--In a series of experiments, Kjellstrand et al. (1981; 1983a,b) evaluated the effects of TCI exposure on body and organ weights, and plasma butyrylcholinesterase in Sprague-Dawley rats, various mouse strains, and mongolian gerbils. Kjellstrand et al. (1981) found suggestive evidence that rats, NMRI mice, and gerbils (consisting of pooled males and females) showed relative and absolute liver weight gain after continuous exposure to 150 ppm (807 mg/m^3) for up to 30 days. The effect seemed to be more pronounced in mice. During postexposure, relative liver weights decreased. Study design limitations hampered evaluation of the statistical significance of results; body weight gains were determined by linear regression.

In a follow-up experiment, Kjellstrand et al. (1983a) obtained more convincing evidence that exposure to 150 ppm (807 mg/m^3) for 30 days caused liver weight gain in various mouse strains (wild, C57BL, DBA, B6CBA, A/sn, and NZB). Plasma butyrylcholinesterase, a liver-produced enzyme of unknown function, also showed statistically significant increased activity in males of all strains and in females of strains A/sn and NZB.

In a further extension of their studies, Kjellstrand et al. (1983b) exposed male and female NMRI mice continuously (up to 120 days) to levels from 37 to 300 ppm (200 to 1614 mg/m^3) and intermittently to 225 to 3600 ppm (1233 to 19,728 mg/m^3). Regardless of the exposure schedule, enzyme activity was highest in male mice under all exposure schedules. Peak activity was reached about day 30. Activity in female mice was largely unaffected by TCI exposure. Liver weights in both sexes increased under either continuous or intermittent exposure. Peak increase in liver weight during 150 ppm exposure occurred at about day 30.

Light microscopy revealed distinct changes in liver morphology at exposure levels as low as 37 ppm (200 mg/m^3). Rehabilitation during postexposure revealed that the liver became histologically similar to air-exposed controls. TCI exposure had little effect on body weight and spleen weight. While kidney weight increased during exposure, weight gain decreased during postexposure rehabilitation. It was concluded that TCI in this species induced a transient abnormal state but no liver damage. It was noted that Shimada and Yoshida (1980) proposed that elevations in butyrylcholinesterase activity may represent fatty metamorphosis or toxic damage to the liver. The finding that butyrylcholinesterase activity is largely confined to male mice and that tumor induction appears similarly sex-specific to males (NCI, 1976) suggests that further investigation of the role of butyrylcholinesterase in metabolism warrants attention.

Other inhalation studies (see Table 5-1) have been more limited in scope, and findings were generally unremarkable (Gehring, 1958; Nowell et al., 1954;

TABLE 5-1. EFFECTS OF TCI EXPOSURE ON EXPERIMENTAL ANIMALS

Mode of Exposure	Exposure Dose	Schedule	Effects	Reference
Inhalation				
Mouse (NMRI)	150 ppm	Continuous up to 30 days for most groups	Analysis of b.w. gain limited in mice by poor study design. Relative liver weight increased and then decreased during rehabilitation.	Kjellstrand et al., 1981
Mongolian gerbil	150 ppm	Continuous for 30 days	No strong evidence for b.w. gain or loss. Small but significant relative liver weight increases. Kidney weight increases significant for males and females.	
Rat (S-D)	150 ppm	Continuous for 30 days	Only increased b.w. for some female rats. Small but significant increases in relative liver weight.	
Mouse (wild, C57BL, DBA, B6CBA, A/sn, NZB, NMRI)	150 ppm	Continuous for 30 days	All strains showed large increases in liver weight. Small changes in kidney and spleen weight. Plasma butyrylcholinesterase activity increased in males of all strains.	Kjellstrand et al., 1983a
Mouse (NMRI)	37, 75, 150, and 300 ppm	Continuous for 30 days (also for 120 days at 150 ppm)	Males exposed to 300 ppm had 3.5 increase in butyrylcholinesterase; females had slight changes at any level. Relative liver weight was linear function of concentration ranged. Increase dropped when body weight dropped. Body weight gain was less than controls. Kidney weight increased more in males (significant at 75 ppm). Spleen weight unchanged. Butyrylcholinesterase activity peaked at day 30; returned to normal during postexposure. Liver weight increase also peaked near day 30. Liver weight returned to near normal during postexposure.	Kjellstrand et al., 1983b

TABLE 5-1. (Continued)

Mode of Exposure	Exposure Dose	Schedule	Effects	Reference
			After 30 days at 150 ppm, liver cells larger and had fine vacuolization of cytoplasm. Kuppfer cells increased in cellular and nuclear size; connective tissue infiltrated with inflammatory cells. No bile stasis. During postexposure, morphology similar to controls.	
Mouse (NMRI)	225, 450, 900, 1800, and 3600 ppm	30 days (hours/day x days/week) 16x7 8x7 4x7 2x7 1x7 respectively	Butyrylcholinesterase significantly increased in males by average exposure of 150 ppm irrespective of pulse. Females unaffected. Liver, body, and kidney weights increase unaffected by pulse. Motor activity increased at 3600 ppm but decreased at 900 ppm. Liver morphological differences more pronounced than with continuous exposure. Mitoses more frequent in groups exposed longer intervals.	Kjellstrand et al., 1983b
Mouse	5500 ppm	Acute	No increase in SGPT; histopathology not performed.	Gehring, 1958
Dog, rabbit, rat	500 to 1000 ppm	18 hr/day, 90 days	No gross changes in liver, renal, or blood functions.	Nowill et al., 1954
Rat	15,000 ppm	Intermittent	No liver or kidney damage. Histopathology not performed.	Utesch et al., 1981
Rat, guinea pig, dog, rabbit, monkey	35 and 700 ppm	8 hr/day, 5 days/ week for 30 exposures over a 6-week period.	No signs of hepatotoxicity. No mortality. 15 rats and guinea pigs, 3 rabbits and monkeys, and 2 dogs exposed at each dose. No changes in liver enzymes.	Pendergast et al., 1967

TABLE 5-1. (Continued)

Mode of Exposure	Exposure Dose	Schedule	Effects	Reference
Albino rat	Anesthetic	2 hr	No signs of parenchymal liver damage. Distribution of acid phosphatase-positive granules irregular and diminished in number, 24 hr after exposure.	Ramadan and Ramadan, 1969
Dog	750 ppm	8 hr/day, 6 days/week 3 weeks	Glycogen depletion and hydropic parenchymatous liver degeneration observed.	Seifter, 1944
	500 to 600 ppm	6 hr/day, 5 days/week 8 weeks	Test mixture may have contained contaminants.	
Rat	1% TCI	2 hr	Glutathione levels decreased during TCI exposure of phenobarbital-treated rats. Rebounded during postexposure.	Moslen et al., 1977
Gavage				
Male mice	0, 250, 500, 1200, or 2400 mg/kg	5 days/week 3 weeks	Dose-related changes characteristic of hepatocellular hypertrophy at all dose levels. Slight changes at lowest dose, increased liver weight at 500 mg/kg, severe centrilobular hepatocyte swelling, giant cell inflammation, and mineralized cells at highest dose. No effect on kidney or body weight.	Stott et al., 1982
Rat	0 and 1100 mg/kg	?	Slightly increased liver weight but no histopathological changes. No effect on kidney or body weight.	Stott et al., 1982
Swiss mice (mixed sexes)	360 to 4300 mg/kg	1x	Fatty infiltration. Minimum lethal dose: 2920 mg/kg. Minimum hepatotoxic dose: 720 mg/kg.	Jones et al., 1958

TABLE 5-1. (Continued)

Mode of Exposure	Exposure Dose	Schedule	Effects	Reference
Rat	85 mg/kg and 340 mg/kg	ix	No effect on liver weight or triglycerides. Effects only if pretreated with phenobarbital.	Danni et al., 1981
Weanling CD-1 ICR outbred albino mice (mixed sexes)	24 and 240 mg/kg	14 days	Apparent dose-related increase in liver weight.	Tucker et al., 1982
	730 mg/kg (day 0) 1460 mg/kg (day 1) 2910 mg/kg (day 3) 1460 mg/kg (days 4, 5) sacrifice (day 6)	5 days 2x 2x 2x 1x	Aniline hydroxylase significantly elevated and aminopyrene N-demethylase significantly decreased. Oral LD_{50} in females: 1839 to 3779 mg/kg; in males: 2065 to 2771 mg/kg.	
Subcutaneous				
Rat	500 mg/kg	1x	SGOT elevated within 12 to 16 hr. Correlated with hepatocellular damage seen in histopathology.	Wirtschafter and Cronyn, 1964
Drinking Water				
Weanling CD-1 ICR outbred albino mice (mixed sexes)	0.1, 1.0, 2.5, and 5.0 mg/ml in 1% Emulphor	4 and 6 mo	Decreased water consumption in females at highest dose and in males at two highest doses. Gross pathology unremarkable. Significant increase in kidney weight (males) at highest dose at 6 mo and in females at 4 and 6 mo. Elevated protein and ketone levels in urine in high-dose females (6 mo) and two highest doses for males (6 mo). Decreased RBC count in high-dose males (4 and 6 mo), decreased leukocyte counts, altered coagulation values in males (4 and 6 mo), and shortened prothrombin time (females, 6 mo).	Tucker et al., 1982

Utesch, 1981; Pendergast et al., 1967; Ramadan and Ramadan, 1969). In many of these studies, histopathology was not performed.

5.4.2.2 Gavage--Stott et al. (1981) found dose-related changes characteristic of hepatocellular hypertrophy at all dosage levels in mice. This study included dosages comparable to those used in the National Cancer Institute (NCI, 1976) bioassay for carcinogenicity of TCI by gavage in B6C3F1 mice and Osborne-Mendel rats (see Chapter 7). In the Stott et al. study, male mice were given 0, 250, 500, 1200, or 2400 mg TCI/kg body weight/day, 5 days/week, for 3 weeks. The rats received 0 or 1100 mg TCI/body weight/day. Rats did not show histopathological changes but had slightly increased liver weights. The authors suggest that the greater sensitivity of mice reflects their greater capacity for metabolic activation. The species difference could very well be due to metabolic saturation in the rat (chapter 4).

Relative liver weight and hepatic triglyceride content were used as parameters in measuring liver injury in the study by Danni et al. (1981). No injury in Wistar rats at dose levels of 85 mg/kg and 340 mg/kg was observed unless the animals were first pretreated with phenobarbital. It was noted by Moslen et al. (1977) and by Adams et al. (1951) that acute liver necrosis is produced only when TCI inhalation is preceded by pretreatment with phenobarbital. In phenobarbital-treated rats, hepatic glutathione levels decreased during TCI exposure but rebounded during the postexposure period (Moslen et al., 1977).

In a 14-day gavage study with weanling CD-1 and ICR outbred albino male and female mice, the only effect noted was an apparent dose-related increase in liver weight. Doses given were 24 and 240 mg/kg (Tucker et al., 1982).

5.4.2.3 Drinking Water--Tucker et al. (1982) evaluated the toxicity of TCI to male and female mice administered TCI in a 1 percent solution of Emulphor. Results of gross pathology on mice sacrificed at 4 and 6 months were unremarkable. Dose levels were 0.1, 1.0, 2.5, and 5.0 mg TCI/ml. Decreased water consumption was evident in females at the highest dose level and in males at the two highest dose levels, compared to vehicle controls. Urinalysis and hematological findings are indicated in Table 5-1.

5.5 IMMUNOLOGICAL AND HEMATOLOGICAL EFFECTS

The immunological status of male and female CD-1 mice exposed to TCI in drinking water for 4 and 6 months was investigated by Sanders et al. (1982).

Details of exposure and discussion of toxicological effects noted were reported separately by Tucker et al. (1982) (see Section 5.4.2). In the 14-day range-finding study, male CD-1 mice had been dosed by p.o. gavage daily with 24 or 240 mg/kg TCI. Although the humoral immune response to sheep RBC was not significantly different from control, the cell-mediated response was inhibited in a dose-related manner. To determine the immune response over longer periods of time, both male and female mice were exposed to TCI (0.1, 1.0, 2.5, and 5 mg/ml) in drinking water for 4 and 6 months. Parameters evaluated included humoral immunity, cell-mediated immunity, lymphocyte responsiveness, bone marrow function, and macrophage function. It was observed that male mice were relatively unaffected after both 4 and 6 months of exposure compared to results in the 14-day study. On the other hand, females were affected, particularly in the 4-mo exposure. Humoral immunity was inhibited only at the highest TCI concentrations (2.5 and 5 mg/ml), whereas cell-mediated immunity and bone marrow stem cell colonization were inhibited at all four concentrations of TCI. The proliferation capacity of the lymphocyte was not affected when challenged with T-cell mitogens.

Hobara et al. (1984) investigated the acute effects of TCI on hematologic parameters in anesthetized, mature, cross-bred dogs. A dose-related decrease in leukocyte counts was observed over the TCI exposure range of 0 to 700 ppm (0 to 3,766 mg/m^3). Five dogs were exposed to each of the following treatments for one hour: 200, 500, 700, 1000, 1500, and 2000 ppm (1026, 2690, 5380, 8070, and 10,760 mg/m^3). In addition, five dogs were exposed to 700 ppm for one hour. Leukocyte counts decreased at the start of exposure and at the end of exposure reached minimum values. During postexposure, values gradually recovered. No significant changes were observed in erythrocyte counts, hematocrit values, or thrombocyte counts. It was the authors' opinion that the decrease in leukocytes reflects the sequestering of the leukocytes in tissues, as a defensive mechanism.

Koizumi et al. (1984) found that δ-aminolevulinic acid dehydratase (ALA-D), an enzyme important in heme synthesis in erythrocytes and liver, was inhibited in rats exposed to 50, 400, or 800 ppm (269, 2152, or 4304 mg/m^3) TCI continuously for 48 or 240 hours. A dose-response relationship was observed. TCA and TCE were not inhibitory *in vitro*. Inhibition of ALA-D (38 percent or 48 percent of the control in liver or in blood, respectively) observed after

240 hours of exposure to 400 ppm (2152 mg/m^3) was accompanied by the significant elevation of δ-aminolevulinic acid synthetase (186 percent of control) in liver and an increase in excretion of ALA in urine (142 percent of control). No effects on weight, liver, or other hematological parameters were observed.

Fujita et al. (1984) also demonstrated an inhibition of ALA-D in rats exposed to TCI for 240 hours. Exposure levels were 50, 377, and 754 ppm (270, 2140, and 4128 mg/m^3). TCI inhibited the holoenzyme only when incubated with a mixed-function oxidase system, suggesting that metabolites play a role. The apoenzyme (without the zinc cofactor) was inhibited in a dose-dependent manner. After exposure to 377 ppm (2140 mg/m^3), urinary ALA increased to 142 percent of control. In livers of exposed rats, cytochrome P-450 concentrations and heme saturation of tryptophan pyrrolase were reduced. Both measures are indices of the free heme pool. Lead levels in blood were normal, indicating that lead was not responsible for the inhibition. Inhibition of ALA-D in the liver was observed at the lowest dose and after 48 hours exposure to TCI.

5.6 DERMAL EFFECTS

Industrial use of TCI is often associated with dermatological problems (Bauer and Rabens, 1974). These include reddening and dermatographic skin burns on contact (Maloof, 1949), generalized dermatitis resulting from contact with the vapor (McBirney, 1954; Conde-Salazar et al., 1983), and possible scleroderma (Reinl, 1957; Walder, 1983). However, irritations, burns, and rashes are usually the result of direct skin contact with concentrated solvent and are, therefore, probably limited to effects secondary to the solvent. No effects have been reported with exposure to TCI in dilute aqueous solutions.

Phoon et al. (1984) reported recently on severe erythema (Stevens-Johnson syndrome) in five individuals occupationally exposed to TCI through vapor and direct dermal contact. Duration of exposure ranged from 2 to 5 weeks and exposure levels were estimated to be between 9 and 169 ppm (50 and 912 mg/m^3). The common occupational history and similarities in condition suggest that TCI may have been a causative factor in eliciting a hypersensitive response.

5.7 MODIFICATION OF TOXICITY: SYNERGISM AND ANTAGONISM

The manifestation of adverse effects of TCI appears to depend, in part, on its metabolic products. Therefore, drugs and chemicals that enhance or inhibit the metabolism of TCI can have a corresponding effect on the toxicity of TCI. Reynolds (1976) and Moslen et al. (1977) have reported that the hepatotoxic potential of TCI can be greatly enhanced by prior treatment with drugs or chemicals that induce components of the liver mixed-function oxidase (MFO) system. Carlson (1974) demonstrated that pretreatment of male albino rats with phenobarbital (50 mg/kg i.p.) or 3-methylcholanthrene (40 mg/kg) exacerbated TCI-induced liver damage. There was massive necrosis and a three-fold increase in SGOT and SGPT levels. Groups of animals were exposed to 6900 ppm (37,122 mg/m^3) for 2 hours and 10,400 ppm (55,952 mg/m^3) for 2 hours. This was confirmed in a later study of Moslen et al. (1977), who found that TCI induced morphological changes in the liver of animals treated with only phenobarbital. Reynolds (1976) also found acute liver injury following a 2-hr exposure to 1 percent TCI in animals pretreated with other inducers of MFO, including chlorinated biphenyls (Arochlor 1245). These findings suggest that a metabolite(s) of TCI contributes to the toxicity of the compound. In this regard, Mikiskova and Mikiska (1966) have found that trichloroethanol, a metabolite of TCI, was three to five times more effective in inducing changes in EKG and EEG.

Masuda and Nakayama (1982) found that TCI produced moderate increases in plasma glutamine pyruvate transminase activity in male SPF mice given an i.p. dose of 2 ml/kg (dissolved in olive oil). Diethyldithiocarbamate trihydrate and CS_2, when given to the mice orally, 30 minutes before TCI, exerted a protective effect, in that the increase in plasma glutamine-pyruvate transaminase was lessened. Diethyldithiocarbamate was effective at each of the three dose levels used (10, 30, and 100 mg/kg). In controls given only TCI, no marked pathological alterations were reported upon histological examinations. CS_2, at levels of 3, 10, and 30 mg/kg, acted similarly to diethyldithiocarbamate in suppressing the TCI-associated increase in plasma glutamate-pyruvate transaminase. The authors concluded that the protective mechanisms of diethyldithiocarbamate and CS_2 may involve their interference with metabolic activation of a variety of hepatotoxins.

Novakova et al. (1981) reported that an open-ended _in vitro_ rat liver perfusion system could be used to demonstrate the effect of TCI and other halogenated solvents on liver enzyme activities. TCI in such a system was reported to increase the activities of aspartate aminotransferase, alamine aminotransferase, alkaline phosphatase, and ornithine carbamoyltransferase.

Pessayre et al. (1982) have obtained evidence that TCI is an effective potentiator of hepatotoxic effects of CCl_4 _in vivo_ in rats. While liver histology was normal 24 hours after i.p. administration of TCI (1 ml/kg) and also after administration of 64 µl/kg CCl_4, both solvents coadministered produced extensive centrilobular necrosis. TCI given _in vitro_ 5 hours prior to CCl_4 increased CCl_4-induced lipid peroxidation. Similarly, coadministration markedly increased serum alamine amino transferase and decreased hepatic cytochrome P_{450} concentrations. Coadministration also decreased hepatic glutathione levels. It was concluded that the toxicologic effects produced by the concomitant administration of TCI and a small dose of CCl_4 resembled those produced by a high dose of CCl_4, a finding consistent with the view that TCI potentiated the hepatotoxicity of CCl_4. It was further concluded that this potentiation may be the result of increased CCl_4-induced rate of lipid peroxidation. Since stable metabolites of TCI failed to stimulate such peroxidation, it was apparently mediated by TCI itself.

The effect of ethanol and other substances upon the manifestation of effects of subsequent TCI exposure in animals has been studied by a number of investigators.

One of the earliest studies was that of Cornish and Adefuin (1966). Male Sprague-Dawley rats were orally pretreated with ethanol (50 percent in water) in a single dose, on the basis of 5 g/kg body weight. Sixteen to eighteen hours after pretreatment, each group (6 rats) was exposed to TCI in a dynamic exposure chamber. Exposure levels were 100, 2000, 5000, and 10,000 ppm (538, 10,760, 26,900, and 53,800 mg/m^3) TCI (3 rats in the highest exposure category died). Animals ingesting ethanol prior to exposure to TCI showed a statistically significant ($p < 0.05$) increase in SGOT, SGPT, and serum isocitric dehydrogenase (SICD) at 5000 ppm (26,900 mg/m^3). After exposure to 5000 ppm (26,900 mg/m^3) for 4 hours, the SGOT of animals pretreated with ethanol was about 20 times the control level, whereas exposed rats not receiving ethanol had normal SGOT values. Animals exposed to 2000 ppm (10,760 mg/m^3) or less for 4 hours showed no marked alteration of serum enzyme levels. Neither ethanol-treated nor nontreated rats showed any alterations of serum enzyme levels when exposed for 8 hours to 100 ppm (538 mg/m^3). All rats exposed to 5000 ppm (26,900 mg/m^3) TCI showed slight to widespread degenerative lipid infiltration of the liver. Ethanol-treated rats (before TCI exposure) exhibited early centrilobular necrosis with slight acute inflammatory cellular reaction.

Muller et al. (1975) proposed that TCI and ethanol undergo similar metabolic steps, thus allowing for a variety of interactions, depending on dose levels and the sequence of exposure. When simultaneous exposure occurs, TCI and ethanol may compete for both alcohol dehydrogenase and microsomal mixed-function oxidase. Intolerance of TCI-exposed workers to alcohol has been described by Defalque (1961). It is often manifested as red flushes on the face and upper parts of the body and has been referred to as "degreasers flush." Stewart et al. (1974) observed this effect in seven individuals given as little as 0.5 ml ethanol/kg during exposure to 20, 100, and 200 ppm (108, 538, 1076 mg/m^3) TCI for 1, 3, or 7.5 hours. Alcohol intolerance among TCI-exposed workers has also been described by Bardodej et al. (1952) and Bardodej and Vyskocil (1956).

Traiger and Plaa (1974) reported that isopropyl alcohol or acetone, administered to mice in an 18-hr pretreatment, potentiated the hepatotoxic response to two doses of TCI, 11.1 mmol/kg and 16.7 mmol/kg.

Cornish et al. (1977) found that rats given i.p. doses of 0.5, 1.0, and 2.0 ml TCI/kg showed progressively increasing levels of SGOT. Pretreatment with either phenobarbital or ethanol or both had no effect on this response. This lack of response with ethanol is in contrast to the earlier study by Cornish and Adefuin (1966) but may reflect different routes of exposure.

Utesch et al. (1981), in developing their animal model for solvent abuse (see Section 5.4.2), found that pretreatment of Wistar-Munich rats with ethanol exacerbated the CNS-depressant effects of high doses of TCI. A group of rats was given 5 ml/kg ethanol orally 1 hour before initiation of the TCI inhalation regimen: 15 exposures cycles in a 5-hour period; each cycle consisted of 5 minutes of inhalation of 15,000 ppm (80,700 mg/m^3) TCI followed by a 15-min solvent-free interval. Ethanol was shown to cause significant prolongation of the animals' ability to regain the righting reflex after the second and each subsequent TCI exposure. Administration of ethanol alone did not result in an increase in levels of SGOT or SGPT in histopathologic alterations of the liver or kidneys. No elevations in SGPT were seen in any of the groups that received both alcohol and TCI. Histopathologic examination of the liver and kidneys revealed no evidence of chemically induced injury in any animals. Fasting for 16 hours before the 5-hr intermittent TCI exposure regimen had no apparent effect on the combined CNS-depressant action of ethanol and TCI. No histopathological effects were observed in the livers or kidneys of these animals.

Moslen et al. (1977) also reported that fasting had little effect on SGOT and SGPT levels in rats which inhaled 10,000 ppm (53,800 mg/m^3) TCI continuously for 2 hours. However, pretreatment of rats with phenobarbital in combination with fasting produced significant elevations in the levels of these serum enzymes.

Cornish et al. (1973) reported that phenobarbital pretreatment produced no additional effect on SGOT above that produced by 1.0 and 2.0 ml TCI/kg, given i.p. to Sprague-Dawley rats. Lower doses of TCI, 0.5 and 0.3 ml/kg, had no effect on SGOT.

Pretreatment of male albino rats with either phenobarbital or 3-methyl cholanthrene was shown by Carlson (1974) to potentiate the hepatotoxicity of TCI. Rats were injected with either 50 mg phenobarbital/kg i.p. for 4 days or 3-methylcholanthrene in corn oil, 40 mg/kg i.p. for 2 days. Animals were exposed to TCI 24 hours after the last dose of phenobarbital or 48 hours after the last dose of 3-methylcholanthrene in a dynamic inhalation chamber. Rats were sacrificed 22 hours after exposure. Phenobarbital pretreatment increased SGOT and SGPT three-fold above TCI controls. TCI exposure was 10,400 ppm (55,952 mg/m^3) for 2 hours. Similarly, 3-methylcholanthrene increased SGOT and SGPT.

5.8 SUMMARY OF TOXICOLOGICAL EFFECTS

There is no reliable information concerning the toxicological effects in humans of chronic exposure to levels of TCI below the TLV® (50 ppm). Based upon acute human overexposure information and limited animal testing, it is unlikely that chronic exposure to TCI at levels found or expected in ambient air would result in liver or kidney damage. Such damage has not been generally found even when exposure greatly exceeds the TLV®.

The first sign likely to be observed upon excessive exposure to TCI is central nervous system (CNS) dysfunction. Psychomotor function and subjective complaints which have been studied in short-term controlled human studies, as well as data from accidental exposure and epidemiological studies, indicate that nervous system function probably is affected by TCI concentrations ranging from 200 to 500 ppm (1076 to 2690 mg/m^3). Dose-response relationships for adverse health effects associated with TCI have not been established in man or experimental animals.

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6. TERATOGENICITY, EMBRYOTOXICITY, AND REPRODUCTIVE EFFECTS

Because of its widespread use, TCI has been studied for teratogenic potential. Teratology studies have been performed in rats, mice, and rabbits using doses of TCI which, in some studies, produced slight signs of maternal toxicity. Also, TCI is known to be metabolized in the maternal host by hepatic metabolizing enzymes (and possibly also in the fetal liver) to chloral hydrate and then to trichloroethanol and trichloroacetic acid. These metabolites, particularly trichloroethanol, have also been shown to readily cross the human placenta into the fetal circulation and amniotic fluid (Bernstine and Meyer, 1953; Bernstine et al., 1954) and also into the breast milk of nursing mothers (Bernstine et al., 1956). There is little information available on the fetotoxic and teratogenic potential of these metabolites. Chloral hydrate and its metabolite, trichloroethanol, have been in common medical use for a great many years as hypnotics, including use during pregnancy, but with no reported adverse teratogenic, fetotoxic, or reproductive effects (Goodman and Gilman, 1980). Trichloroethanol has been administered to three animal species at various stages of pregnancy, at levels as high as 700 mg/kg/day, without dose-related effects (Physicians Desk Reference, 1981). Other studies in avian embryo systems (Fink, 1968; Elovaara et al., 1979) have indicated that TCI disrupts embryogenesis in a dose-related manner. Another study in the avian embryo system (Bross et al., 1983) indicated that TCI is embryotoxic and teratogenic in chick embryos. However, because administration of TCI directly into the air space of chicken embryo is not comparable to administration of dose to animals with a placenta, it is not possible to interpret this result in relationship to the potential of TCI to cause adverse effects in animals or humans. The following discussion of studies subscribes to the basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" as summarized and stated by the U.S. Environmental Protection Agency (1980):

> Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring *in utero*. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis edema, and increased

incidence of supernumerary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.

In view of the spectrum of effects at issue, the Agency suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to in utero exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.

Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up." Unless a series of specific kidney function tests are performed on the neonate, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting aftereffects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/ fetal effect in one species might, under a given agent, react in another species in a more serious and irreversible manner.

6.1 ANIMAL STUDIES

6.1.1 Mouse

Schwetz and his associates (Schwetz et al., 1975; Leong et al., 1975) investigated the potential of TCI to adversely affect fetal development in Swiss-Webster mice (Table 6-1). Thirty to forty bred mice were exposed by inhalation to TCI (99.24-percent pure) at an air concentration of 300 ppm (1614 mg/m^3) for 7 hours daily on days 6 through 15 of gestation. The results from two separate trials of 12 and 18 pregnant animals were reported. The first day of pregnancy was designated as the first day a vaginal plug was observed. Concurrent pregnant control mice (30) were exposed to filtered air. The mice were observed daily throughout pregnancy, and maternal body weights

TABLE 6-1. SUMMARY OF ANIMAL STUDIES OF FETOTOXIC AND TERATOGENIC POTENTIAL OF TCI

	TCI purity	Species	Conditions (mode of administration, dosage and duration of exposure)	Measures	Results
Fink (1968)	Unknown	Chick embryo	Vapor exposure, 10,000 ppm	Mortality anomalies	Increase Slight increase
Elovaara (1979)	Reagent grade	Chick embryo	Injected; 5 - 100 μmole per egg in 25 μl olive oil	LD_{50}	50 - 100 μmole/egg (16 % malformations in total survivors)
Schwetz et al. (1975)	Technical 99.24 % TCI 0.76 % stabilizers and impurities (Neu-tri)	S - D Rat ♀ S - W mouse ♀	Inhalation; 300 ppm, 7h/d on 6 - 15-d gestation	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)
Bell (1977)	Technical (Trichlor 132)	CR - SD rat ♀	Inhalation; 300 ppm, 6h/d on 6 - 15-d gestation	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)
Dorfmueller et al. (1979)	Technical 99 %+ TCI 0.2 % epichlorohydrin (Neu-tri)	Rat ♀ (Long-Evans)	Inhalation, 1800 ppm a) Premating: 6h/d, 5d/wk for 2 wk b) Premating for 2 wk + first 20-d gestation, daily c) First 20-d gestation, daily	Embryo toxicity, teratogenicity, offspring behavioral evaluation (maternal toxicity)	+ - (+)
Beliles et al. (1980)	Technical 99.9 %	CR - SD Rat ♀ Rabbit ♀	Inhalation; 500 ppm, 7h/d, 5d/wk Premating 3 wk + first 18-d gestation, daily (rat) + first 21-d gestation, daily (rabbit)	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)

were recorded on days 6, 10, 16 and 18 of gestation as an index of maternal toxicity. Following caesarean section on day 18, the fetuses were weighed, measured, (crown-rump length), sexed, and then examined for external anomalies. One-half of the fetuses were examined for soft tissue malformation, using free-hand sectioning, and one-half of the fetuses were cleaned, stained, and examined for skeletal malformations. One fetus in each litter was subjected to microscopic examination.

Exposure to TCI had no effect on either the mean, absolute, or relative weight of the liver of the maternal animals when measured at the time of caesarean section. Exposure had no effect on the average number of implantation sites per litter, litter size, the incidence of fetal resorptions, fetal sex ratios, or fetal body measurements. The incidence of gross anomalies observed by external examination was not significantly greater than among control litters. TCI exposure had no effect on the incidence of skeletal anomalies, and microscopic examination revealed no abnormalities of organs, tissues, or cells. These investigators concluded that 300 ppm (1614 mg/m^3) TCI caused no significant maternal, embryonal or fetal toxicity, and that TCI was not teratogenic in Swiss-Webster mice at this dose level.

6.1.2 Rat

Schwetz et al. (1975) carried out a study in Sprague-Dawley rats. The design of the study was similar to their protocol for mice described above. TCI (300 ppm; 1614 mg/m^3) was administered by inhalation, 7 h daily, to 18 pregnant rats during organogenesis, the period when the greatest susceptibility to teratogenic effect is present (days 6 to 15 of gestation). Controls (30) were provided filtered air. Day 0 of gestation was defined as the day spermatozoa were observed in vaginal smears. The animals were killed on day 21 of gestation, caesarean sections were performed, and fetuses in each litter were examined for external malformations. One-half of the fetuses in each litter were then examined for soft tissue malformations, and the other half examined for skeletal malformations. One fetus from each litter was randomly selected for serial sectioning and histological evaluation. Schwetz et al. (1975) reported a small but statistically significant inhibition of maternal body weight gain when dams were exposed to 300 ppm (1614 mg/m^3) TCI. However, this dose had no effect on either the mean, absolute, or relative weight of the maternal host livers when measured at the time of caesarean section. Exposure to TCI had no effect on the average number of implantation sites per litter,

litter size, the incidence of fetal resorptions, fetal sex ratios, or fetal body measurements. No increased incidence of soft tissue or skeletal anomalies was observed, and histopathological examination revealed no abnormalities of organs, tissues, or cells. Schwetz et al. (1975) concluded that 300 ppm (1614 mg/m^3) TCI causes little or no significant maternal, embryonal or fetal toxicity in Sprague-Dawley rats.

Bell (1977) reported a similar study, conducted by Industrial Bio-Test Lab. Inc. (Northbrook, Ill.), on the teratogenic and fetal toxic effects of TCI on Charles River rats. TCI (300 ppm; 1614 mg/m^3) was administered to 17 dams by inhalation exposure, 6 h per day, from day 6 through day 15 of gestation. Control pregnant animals (21) were killed on day 20 of gestation. Caesarean sections were performed and the number of fetuses, implantation sites, resorption sites, and corpora lutea were counted. Viable fetuses were counted, and all fetuses were removed and weighed. External examination of the fetuses was made for gross anomalies. Two-thirds of the fetuses from each litter were examined for skeletal development, and the remaining one-third of the fetuses were evaluated for soft tissue abnormalities. The investigators found that 300 ppm (1614 mg/m^3) TCI exposure produced little or no evidence of maternal toxicity, although a small but significant reduction of mean weight gain by gestation day 15, in comparison to control dams, was recorded. There were no mortalities or adverse behavioral reactions, gross fetal anomalies, or differences in mean weights of fetuses exposed to TCI in comparison to unexposed controls. The numbers of corpora lutea, implantation sites, resorption sites, and fetuses were similar among exposed and control dams. No evidence for teratogenicity was observed in fetuses from either exposed or unexposed dams.

Beliles et al. (1980) reported on a study of teratogenic and fetotoxic potential of TCI in Sprague-Dawley rats conducted by Litton Bionetics, Inc. (Kensington, MD.). The experimental design of the study included pregestational as well as gestational exposure (Table 6-2).

Exposure to TCI, 500 ppm (2690 mg/m^3) in air, was 7 h/d, 5 d/wk, during a 3-wk pre-gestational period, and 7 h/d each day for days 0 to 18 and days 6 to 18 of gestation. Positive identification of spermatozoa in the vaginal canal was taken as evidence of mating and designated as day 0 of gestation. The dams were killed on day 20 of gestation; caesarean section was performed, visceral and thoracic organs were examined, and the numbers corpora lutea per dam determined. The maternal livers, kidneys and lungs were weighed. The numbers

TABLE 6-2. EXPERIMENTAL DESIGN OF BELILES et al. STUDY (1980) ON SPRAGUE-DAWLEY RATS

Group	No.	TCI ppm	Days of Exposure (Controls exposed to air only)	
			Pre-gestational	Gestational
1	21	0 (control)	None	0-18
2	16	0 (control)	(5 day/wk) 3 wk	0-18
3	20	500	None	0-18
4	16	500	(5 day/wk) 3 wk	0-18
5	20	500	None	6-18
6	32	500	(5 day/wk) 3 wk	6-18

of implantation sites, live and dead fetuses, and resorption sites were recorded. The fetuses were examined for gross external anomalies and weighed, and crown-rump length and sex were determined. One-half of the fetuses of each litter were sectioned and examined for changes in the soft tissues; the remaining fetuses were examined for skeletal abnormalities.

The findings reported in this study indicate that maternal toxicity was not a factor in data interpretation; means of body weight, liver, kidneys, and lungs of test dams were within normal expected variation of control dams. No deaths occurred in the exposed groups. The investigators concluded that neither the frequency nor character of the macro- or microscopic findings in the treated groups indicated an adverse effect on fetal growth and development, or a teratogenic potential for TCI at the dose level of 500 ppm (2690 mg/m^3).

Dorfmueller et al. (1979) conducted a study of the teratogenic and fetotoxic effects of TCI in Long-Evans rats exposed to a inhalation concentration of 1800 ppm (9684 mg/m^3) in air. Their experimental design was similar to that of Beliles et al. (1980) insofar as the animals were exposed during the pre-gestational period in addition to during gestation (Table 6-3).

The premating exposure was conducted for 6 hr/day, 5 days/wk for 2 weeks, and the gestational exposure was 6 hr/day each day for days 0 to 20. Day 1 of pregnancy was considered to be the day on which sperm from mating were observed. On day 21 of gestation, 15 out of 30 dams/treatment group were sacrificed; maternal livers were removed, weighed, and frozen for later enzyme analysis. Caesarean sections were performed and the number of live, dead, and resorbed

TABLE 6-3. EXPERIMENTAL DESIGN OF DORFMUELLER et al. STUDY (1979) ON LONG-EVANS RATS

Group	No.	TCI (ppm)	Days of Exposure	
			Pre-gestational	Gestational
A	30	1800 ± 200	5 day/wk; 2 wk	1 - 20
B	30	1800 ± 200	5 day/wk; 2 wk	none
C	30	1800 ± 200	none	1 - 20
D	30	none	none	none

fetuses counted. Each fetus was weighed, the sex was recorded, and then examined for external anomalies. Four fetuses from each litter were examined for soft tissue changes, and four fetuses were evaluated for skeletal abnormalities. Livers were removed from the remaining fetuses for measurement of mixed-function oxidase activity.

Postnatal behavioral evaluation was conducted on the offspring of the remaining 15 dams/treatment group which were allowed to litter. At weaning, 2 male and 2 female pups from each litter were randomly selected for observation of activity measurements at 10 days of age (Motility Meter) and 20 and 100 days of age (Activity Cage).

Dorfmueller et al. (1979) found that the female rats exposed to 1800 ppm (9684 mg/m^3) TCI before mating and/or during pregnancy did not exhibit any signs of maternal toxicity. Weight gains throughout pregnancy were normal in relation to filtered air controls, and the relative and absolute maternal liver weights indicated that no significant treatment effects occurred. There was no indication of embryotoxicity; no significant treatment effects or interactions were found in numbers of corpora lutea or implantation sites/litter, fetal body weights, resorbed fetuses/litter, or sex ratios. No significant treatment effects were observed in the analysis of total soft tissue anomalies. However, the incidence of total skeletal anomalies was increased in group C because of an increased incidence of incomplete ossification of the sternum, but when the frequency of this particular anomaly alone was compared in group C versus group D, the investigators found no significant difference. Incomplete ossification of the sternum is thought to be indicative of delays in the general process of skeletal ossification which are not necessarily irreversible. Thus, prenatal exposure to 1800 ppm (9684 mg/m^3) TCI caused an elevation of minor anomalies indicative of development delays in maturation, but not of major malformations. Also, the study indicated no treatment effect on the general activity of the offspring at 10, 20, and 100 days of age. There was, however, a small but statistically significant depression in postnatal weight gains of offspring in the premating exposure groups (A and B), the biological significance of which was not apparent. The offspring are being maintained until 18 months of age to further assess postnatal behavior and transplacental carcinogenicity potential. This study revealed no results indicative of treatment-related maternal toxicity, embryotoxicity, teratogenicity, or significant postnatal behavioral deficits from exposure, pre-gestational and/or gestational, to 1800 ppm (9684 mg/m^3) TCI in air.

6.1.3 Rabbit

Beliles et al (1980) also studied the fetotoxicity and teratogenicity of TCI in the female New Zealand white rabbit. Their experimental design is presented in Table 6-4.

TABLE 6-4. EXPERIMENTAL DESIGN OF BELILES et al. STUDY (1980) ON NEW ZEALAND WHITE RABBITS

Group	Animals/ group	TCI ppm	Days of Exposure Pre-gestational	Days of Exposure Gestational
1	21	0 (control)	None	0-21
2	18	0 (control)	5 day/wk; 3 wk	0-21
3	23	500	None	0-21
4	19	500	5 day/wk; 3 wk	0-21
5	23	500	None	7-21
6	25	500	5 day/wk; 3 wk	7-21

Exposure to TCI, 500 ppm (2690 mg/m^3) in air, was 7 hr/day, 5 day/wk during a 3-week pre-gestational period, and/or daily for days 0 to 21 or days 7 to 21 of gestation. Observed copulation and/or the presence of sperm in the vaginal canal established day 0 of gestation. On day 30 of gestation, the dams were killed, caesarean-sectioned, and the corpora lutea per dam determined. The maternal livers, kidneys, and lungs were removed, weighed, and examined; the remaining visceral and thoracic organs were also examined. The number of uterine implantation sites, live and dead fetuses, and resorption sites were recorded. The fetuses were examined for gross external anomalies and weighed, and then crown-rump length and sex ratios were determined. One-half of the fetuses of each litter were sectioned and examined for changes in the soft tissues; the remaining fetuses were examined for skeletal abnormalities.

The number of deaths in the TCI-treated groups was not significantly greater than among controls. Deaths occurring during the experiment account for the unequal numbers of animals in the groups. The mean body weights during pre-gestational and gestational periods were determined, and there were no remarkable differences between control and treated rabbits.

This study provided no evidence of maternal toxicity from 500 ppm (2690 mg/m^3) exposure to TCI. Means of body weight, liver, and lungs were within normal expected variation of control dams. A small increase in means of kidney weights for groups 2, 4, and 6 (compared to group 1) was found, but these increases were judged to be unrelated to treatment. No difference was observed in the mean weight of fetuses exposed to TCI in comparison to those of unexposed controls. The numbers of corpora lutea, implantation sites, resorption sites, fetuses and sex ratio were similar among exposed and control dams.

Gross examination of fetuses, with regard to external morphology and internal abnormalities, revealed no unusual changes except for hydrocephalus in 4 fetuses of 2 litters of group 3. This anomaly occurred only in the one treatment group 3 and the malformation was not observed in the litters of rabbits that were exposed to 500 ppm (2690 mg/m^3) TCI during a 3-week pre-gestational period plus the same gestational period (Group 4). The authors confirmed the anomaly as an external hydrocephalus, which, in their experience, does not occur in untreated control rabbits. They, therefore, felt that, although not statistically significant, this finding could not be discounted entirely as occurring by chance. It is not known if the increase in external hydrocephalus observed in this study is a significant observation of a rare biological anomaly. Additional studies would be needed to strengthen this conclusion.

Examination of the fetuses for soft tissue anomalies showed no changes (except external hydrocephalus discussed above) that were not frequently observed in 30-day-old rabbit fetuses of the strain and source used in the study. Skeletal examination revealed no unusual features, except for an increased incidence of changes related to retarded bone ossification, which were not considered malformations as such. Neither frequency nor the character of these changes in the treated groups indicated an adverse effect on fetal growth and development or of a teratogenic potential. In summary, this study in rabbits exposed to 500 ppm (2690 mg/m^3) TCI revealed little or no evidence of maternal toxicity or embryotoxicity; the authors did report the occurrence of hydrocephalus in a few fetuses of one of the study groups, but no definitive conclusion was made regarding this occurrence. A definite conclusion concerning this effect cannot be determined without additional studies that would strengthen this observation.

6.2 FETOTOXICITY IN HUMANS

There are no epidemiologic studies in the literature specifically investigating fetotoxicity or overt birth anomalies that may occur from TCI exposure in the workplace.

Corbett and his associates (1972; 1974) reported an increased incidence of miscarriages in operating room nurses exposed to various general anesthetics (volatile halogenated aliphatic hydrocarbons). TCI was only one of several anesthetics in use, and, therefore, it is impossible to make a specific association of TCI with the miscarriages. Cote (1975) has pointed out weaknesses in this study due to the lack of direct physician examination of the children and a lack of stringent categorization of the types of birth defects. It was suggested by Cote that additional long-term studies be conducted to resolve these issues.

6.3 SUMMARY

Inhalation exposure studies in the mouse and rat by different investigators indicate that TCI does not produce significant signs of developmental toxicity except at levels which affect maternal well-being. One investigator, Beliles et al. (1980), reported the occurrence of an unusual observation in rabbits, hydrocephalus, in a few fetuses from one exposure group. However, this observation was not dose-related and the significance of this finding could not be assessed from this study.

At present, the available studies do not indicate that TCI is toxic to the fetus at levels below the maternally toxic level. Also, no definitive clinical evidence of fetotoxicity or teratogenicity from TCI exposure has been reported. Therefore, the available information does not indicate that the conceptus is uniquely susceptible to the effects of TCI. It should be noted, however, that the potential of TCI to produce external hydrocephalus in rabbits has been discussed by Beliles et al. (1980) and could not be resolved by that study. In addition, TCI has been implicated in producing adverse male reproductive effects (see Chapter 7).

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7. MUTAGENICITY

Trichloroethylene (TCI) has been tested for its ability to cause gene mutations in bacteria, yeast, higher plants, insects, and rodents. It has also been evaluated for its ability to cause chromosome aberrations in insects, rodents, occupationally exposed workers, and in cultured cells, and for its ability to cause other effects indicative of DNA damage (gene conversion, mitotic recombination, sister chromatid exchange, unscheduled DNA synthesis, and DNA alkylation). The results of these tests will be discussed below and summarized in Tables 7-1 to 7-9. Consideration will also be given to studies bearing on the potential of TCI to reach the germinal tissue of mammals.

7.1 GENE MUTATION STUDIES

7.1.1 Prokaryotic Test Systems (Bacteria)

Eight reports were evaluated that concerned testing of TCI for its mutagenic potential in bacteria. Seven used Salmonella typhimurium (Henschler et al., 1977; Simmon et al., 1977; Margard, 1978; Waskell, 1978; Baden et al., 1979; Bartsch et al. 1979; Kline et al., 1982), and one employed Escherichia coli (Greim et al., 1975).

Because of the possibility of mutagenic contaminants in TCI samples, Henschler et al. (1977) chemically analyzed and subsequently tested samples of TCI for mutagenicity. They determined that a sample of technical-grade TCI used in the National Cancer Institute (NCI) carcinogenicity bioassay (NCI, 1976) contained the following impurities (% w/w) by gas chromatography-mass spectrometry analysis: epichlorohydrin, 0.22; 1,2-epoxybutane, 0.20; carbon tetrachloride, 0.05; chloroform, 0.01; 1,1,1-trichloroethane, 0.035; diisobutylene, 0.02; ethylacetate, 0.052; pentanol-2, 0.015; butanol, 0.051. These impurities were tested for mutagenicity with and without S9 mix from PCB-induced Wistar rat livers. In Salmonella typhimurium, two impurities, epichlorohydrin and 1,2-epoxybutane, were found to be mutagenic in tests conducted without activation. Increases of twenty-five and eight times over the spontaneous revertant rates were observed for the two compounds, respectively (extrapolated from Figure 1 of the paper). Negative responses were observed when the tests were repeated using S9 mix. Negative responses were also obtained with carbon tetrachloride, chloroform, and 1,1,1-trichloroethane, both with and without activation, but no data were presented. Similarly, no increased mutation frequency was obtained with "purified" TCI (source and purity not given). Roughly 140 TA100 revertants were observed at each point tested over the entire dose range (from 1.12×10^{-3}M to 1.12 M), both with and without metabolic activation; the spontaneous revertant count was 138 ± 8 (without activation) and 242 ± 10 (with activation) colonies per plate. In their testing, Henschler et al. (1977) added the chemical substances directly to the top agar. No precautions were reported to have been taken to prevent excessive evaporation of the test compound, and, thus, the significance of the negative response obtained with TCI is weakened by the possibility that the material may have evaporated before adequately exposing the indicator cells. This aspect of the study design could not be evaluated because no toxicity data were presented. The positive response after exposure to other volatiles like 1,2-epoxybutane may reflect the potency of these materials compared to TCI. Based on their results, the authors concluded that the carcinogenic activity of technical-grade TCI in the NCI bioassay experiment was probably predominantly, if not exclusively, due to the epoxide contaminants. The basis for reaching these conclusions is inadequate for a number of reasons including flaws in the study design, such as those mentioned above.

Margard (1978) also addressed the hypothesis that the mutagenic activity of technical-grade samples of TCI is due to mutagenic stabilizers rather than TCI by comparing the mutagenic properties of technical and purified grades of TCI in the Salmonella typhimurium/mammalian microsome plate incorporation assay. He used tester strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence and absence of a metabolic activation system (S9 mix) from adult Sprague-Dawley rats induced with Aroclor-1254. Prior to plating, two aliquots of cells were taken. One was diluted and grown on complete media, for determining cell survival, and the other was grown on histidine-deficient plates for selection of his^+ revertants. Cells were then incubated for 48 hours at 37°C and colony counts were made. The technical-grade sample contained 0.08 percent (w/w) epichlorohydrin, 0.02 percent N-methylpyrrole, 0.13 percent N-propyl alcohol, 0.05 percent nitromethane, 0.10 percent 1,1,1-trichloroethane, and 0.45 percent butylene oxide. The purified grade contained no detectable epoxides nor other stabilizing ingredients (written communication with L. Schlossberg, Detrex Chemical Industries, Inc.). A concentration-related increase in the number of revertants was observed, as described in Table 7-1, in strain TA1535, both with and without metabolic activation after exposure to technical-grade TCI. This sample was not mutagenic in strains TA98, TA100, TA1537, and TA1538. Purified TCI was not mutagenic in any strains. TCI levels $\geq$0.1 ml/plate were toxic, as indicated by reduced cell viability (49-86 percent killing). It has been reported that precautions were taken to inhibit evaporation of the test samples during treatment of the cells (Schlossberg, personal communication), but it was not specified in writing what those precautions were. The toxicity data suggest that the cells were exposed to the test material, but it is not possible to determine toxicity adequately in a plate incorporation test. To do this, a liquid suspension assay must be done. Appropriate positive controls were reported and demonstrated that the S9 had metabolic activity. From the data presented, it is not clear how many plates were employed for each dose nor is it clear whether or not replicate testing was performed. Under the conditions of test, "purified" TCI was not mutagenic.

To overcome the concern that the volatility of certain halogenated hydrocarbons may prevent them from being adequately tested in standard plate incorporation tests, Waskell (1978) investigated the mutagenic properties of commonly used volatile anesthetics, including TCI, and several of their known metabolites in testing conducted in sealed chambers. Salmonella typhimurium strains TA100 and TA98 were used in tests for gene reversion. Anesthetic-grade TCI from Ayerst was tested in closed containers at doses up to 10 percent in the atmosphere. The purity of the sample tested was not reported, but it would have been high, with an approximate minimum purity of 99.5 percent and stabilization by 0.1 percent thymol (personal communication with Ayerst). Holes were punched in the top of the Petri dishes to allow the test vapor access to the bacterial strains. Tests were conducted in the presence and absence of phenobarbital- and PCB-induced Sprague-Dawley rat liver microsomes. No toxicity testing was conducted nor were the actual concentrations of TCI measured in the exposure chamber, so it is not known whether the bacteria received a sufficiently high dose. After a 48-hr exposure, TCI did not produce a mutagenic effect in strains TA100 and TA98, with or without metabolic activation. However, no data were presented to support this conclusion. With respect to the metabolites of TCI, the nonvolatile compound 1,1,1-trichloroacetic acid ($\geq$99.0 percent pure, obtained from Matheson, Coleman, and Bell, personal communication with Matheson, Coleman, and Bell), chloral hydrate (recrystallized from chloroform

TABLE 7-1. MUTAGENICITY OF TECHNICAL-GRADE TCI IN SALMONELLA/MAMMALIAN MICROSOME ASSAY

		Activation			Nonactivation		
		Toxicity		Mutagenicity	Toxicity		Mutagenicity
Tester Strain	Conc. (ml/plate)	Plate Count	Rel. Via.*	No. Rev.†	Plate Count	Rel. Via.*	No. Rev.†
TA1535	1.0						
	0.5						
	0.1	27	0.14	58	22	0.14	58
	0.05	142	0.72	40	122	0.78	42
	0.01	185	0.94	17	149	0.95	21
	0	196	--	24	157	--	23
TA100	1.0						
	0.5						
	0.1	53	0.19	184	66	0.28	173
	0.05	207	0.73	263	167	0.71	181
	0.01	255	0.90	123	232	1.0	136
	0	283	--	167	234	--	160
MUTAGENICITY OF PURIFIED-GRADE TCI							
TA1535	0.1						
	0.5						
	0.1	97	0.39	17	89	0.51	15
	0.05	172	0.70	19	121	0.69	20
	0.01	22	0.90	21	156	0.89	20
	0	246	--	22	175	--	19
TA100	1.0						
	0.5						
	0.1	175	0.55	70	166	0.54	114
	0.05	175	0.55	142	162	0.53	158
	0.01	225	0.71	157	354	0.82	145
	0	317	--	154	305	--	158

*Relative viability of tests compared to the control plates.

†Number of revertants observed (average).

Source: Adapted from Margard, 1978.

six times, to achieve a purity of ≥99.0 percent), and trichloroethanol (purity not determined, personal communication with L. Waskell, V.A. Hospital, San Francisco, CA) were tested at maximum nontoxic quantities in plate incorporation tests. Doses of trichloroethanol at 7.5 mg/plate and trichloroacetic acid at 0.45 mg/plate were not mutagenic in strains TA98 and TA100. Chloral hydrate at 10 mg/plate increased the number of TA100 revertants above control (200) by 121 with metabolic activation and 89 without metabolic activation. The data are inconclusive but are suggestive of a weak positive response. These strains were responsive to the positive-control chemicals, diethyl sulfate and 2-aminofluorene.

Further testing was done to evaluate the dose-response of chloral hydrate-induced mutagenesis in strain TA100. The number of revertants per plate in the control groups were 190 and 210 with and without metabolic activation, respectively. Doses of chloral hydrate from 0.5 mg/plate to 10.0 mg/plate did not result in a mutation frequency greater than twofold above the spontaneous level. However, increases in the number of revertants per plate above control values were observed both with and without metabolic activation: 255 to 260 at 0.5 mg; 285 to 290 at 1.0 mg; 300 to 320 at 5.0 mg; and 290 to 310 at 10.0 mg. Although the author reported these increases as statistically significant by the t test ($P < 0.01$), the increases merely suggest, at best, a positive response. The negative response for the induction of gene mutations in bacteria induced by exposure to TCI reported by Waskell (1978) is consistent with the results of Henschler et al. (1977) and Margard (1978). However, equivocal weak-positive responses suggestive of a positive effect have been reported by other investigators using airtight exposure chambers and metabolic activation.

In tests employing Salmonella tester strain TA100, both with and without an exogenous S9 metabolic activation, Bartsch et al. (1979) assessed the mutagenic potential of over 20 chemical substances, including a TCI sample obtained from Merck, Darmstadt, Federal Republic of Germany. The sample was reported to be 99.5 percent pure and contained no detectable amounts of epichlorohydrin or 1,2-epoxybutane at levels ≥1 ppm. Experiments were conducted using a vapor exposure assay in which Petri dishes containing a lawn of TA100 with or without S9 mix were placed, uncovered, in 10-l desiccators for various lengths of time at 37°C, in the dark. Upon completion of the exposure period the compound was removed, and the plates were then covered, inverted, placed in an incubator for an additional 48 hours at 37°C, and scored. TCI was not found to be mutagenic under the conditions used:

Concentration in Air (5% v/v)	Time (hr)	Revertants/plate	
		+S9	-S9
Trichloroethylene			
0		75	70
8	16	85	72
20	16	55 (toxic)	50 (toxic)
Vinyl Bromide			
2	16	240	190
20	16	715	695

When the test conditions were modified, such that plates containing a liver S9 fraction from phenobarbital-treated OF-1 mice, an NADPH-generating system, TA100, and 0.1 mM EDTA were preincubated at 37°C for 4 hours and then exposed to 5 percent TCI vapor in air for 2 hours, a nearly twofold increase over the spontaneous mutation frequency was observed (see table).

Concentration Air (% v/v)	Time (hr)	Revertants/plate	
		+S9	-S9
0		75	70
5*	2	100	80
5*	2†	135	70

*0.1 mM EDTA added to soft agar.

†Preincubated with S9, TA100, and 0.1 mM EDTA for 4 hours, then exposed to TCI vapors.

The results of only one test were presented, but it was stated that near twofold increases over the spontaneous mutation frequency were observed consistently.

Baden et al. (1979) evaluated the mutagenic properties of TCI in Salmonella typhimurium strains TA100 and TA1535, both with and without S9 metabolic activation from PCB-induced male Sprague-Dawley rats. The sample was >99.5 percent pure, contained no detectable epoxides, and was stabilized with 0.1 percent (w/w) thymol (personal communication with M. Kelly, V.A.

Hospital, Palo Alto, CA). Liquid TCI was directly added to bacterial cell cultures for incubation at 37°C for 2 hours, and concentrations in the gas phase above the incubator, which were the same as those in the desiccator experiment described below, were monitored for the first 2 hours of exposure, showing a variation of ≤10 percent. These cultures were plated and incubated for 2 days at 37°C before counting revertant colonies. TCI was not found to be mutagenic in this experiment. However, no monitoring was conducted after the initial 2 hours, and it is not known whether the desired exposure levels were maintained throughout the 2-day incubation period. Toxicity data were not reported, and, therefore, it is not known whether the target cells were actually exposed to a sufficiently high concentration of the test material. In another experiment, reported in the same article, cultures were exposed to vapor concentrations ranging from 0.1 to 10.0 percent (± 10 percent tolerance) in a desiccator for 8 hours at 37°C, with further incubation without treatment for 40 hours before counting of colonies. The experiment was repeated, and each test concentration was evaluated with triplicate assays. TCI vapor produced a less than twofold increase in the number of revertant colonies, to approximately 30 percent above background (149 ± 12, n=15) at concentrations of 1 percent (196 ± 13, n=15), and 3 percent (194 ± 12, n=15) in TA100 with metabolic activation. No thymol control was reported, and the response was not dose-related. At best this study provides only suggestive supportive evidence for mutagenic activity caused by TCI.

Simmon et al. (1977) also reported that reagent-grade TCI, with no detectable epoxides, gave less than a twofold increase in revertant counts in Salmonella typhimurium strain TA100. The sample was 99.85 percent pure, and contaminants included 0.09 percent chloroform, 0.02 percent tetrachloroethylene, 0.01 percent pentachlorobutadiene, and 0.03 percent hexachlorobutene; epoxides were not detected (personal communication with R. Spanggord, SRI International). Plates containing TA100 with and without metabolic activation were exposed to TCI vapor evaporated from a known amount of liquid in a desiccator for 7 hours at 37°C before counting. TCI was detected as mutagenic only with metabolic activation. The number of revertants per plate was increased above the spontaneous control value of about 140, both with and without activation, by TCI treatment as follows: 180, 230, 240, and 200 revertants at 0.5, 1.0, 1.5, and 2.0 ml TCI/9l desiccator, respectively, in the presence of S9 mix from B6C3F1 male mice; and 160, 210, 200, and 170 revertants at 0.5, 1.0, 1.5, and 2.0 ml

TCI/91 desiccator, respectively, in the presence of S9 mix derived from PCB-induced male Sprague-Dawley rats. The revertant counts were extrapolated from Figure 22 of the paper. The results of these assays were reproduced several times (personal communication with V. Simmon, Genex Corp.), but no estimation of the variance of the results (stated to be low) was presented and no data are presented to allow its calculation.

Greim et al. (1975) exposed Escherichia coli strain K12 to 3.3 mM analytical grade TCI during 2 hours of incubation at 37°C in the presence of metabolic activation from phenobarbital-induced male NMRI mouse liver microsomes. A chemical analysis of the test sample was not done by the investigators (written communication with D. Henschler, University of Wurzburg); however, the approximate composition of the TCI product was probably: TCI, 99.9 percent; either $\leq$100 ppm diisopropylamine or $\leq$120 ppm of a combination of diisopropylamine, N-methylpyrrole, and 4-tertbutylphenyl as stabilizers; <500 ppm of other chlorinated hydrocarbons combined (chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,1,2-dichloroethylene [sic] , and tetrachloroethylene); no epoxides present at a detection level of 1 ppm (written communication with Dr. Fries and Dr. Koppe, E. Merck Company, the producer of the sample used in this study). Several loci in E. coli K12 were used to test for mutagenicity: back mutation at three loci, gal^+, arg^+, and nad^+, and the forward mutation MTR system, which yields resistance to 5-methyl-DL-tryptophan. Survival of bacteria on complete medium at the TCI concentration used (3.3 mM) was 76 ± 4 percent. A weak twofold mutagenic effect of TCE, expressed as a percentage of the spontaneous mutation rate, was observed for arg^+, 232 ± 36, but the significance of this response cannot be ascertained because no increase was observed for the other markers; gal^+, 123 ± 23; MTR, 114 ± 18; nad^+, 100. Descriptions of the methodology and the observed results were inadequate. For instance, information on spontaneous mutation frequencies, number of replications, revertant count data, etc. for each test was not reported. Only one dose was used, so it was not possible to obtain dose-response data. Thus, the significance of the results cannot be assessed adequately.

None of the tests described previously clearly showed TCI to be mutagenic. However, the weak, less than twofold, increases in revertant counts observed in several studies, using several samples of TCI and different strains and species of bacteria, suggest that commercially available TCI may be weakly mutagenic to bacteria after metabolic activation. The data for concluding that TCI itself is mutagenic are at best suggestive; the fact that weak, but less than twofold responses were obtained with such samples only after metabolic activation argues against the possibility of the responses being caused by the presence of epoxide stabilizers, because these agents are direct-acting mutagens.

It is important to note that only very weak positive responses (<twofold above the spontaneous level) were obtained with "purified" samples of TCI. In these studies the responses were not always dose-related and were observed only at very high doses (i.e., vapors ranging from 1 to 5 percent v/v). The data are not typical of mutagenic agents in Salmonella, but it is not possible to unequivocally rule out the chance that TCI causes a slight increase in mutant yield. The studies described below discuss the observations from tests with other organisms.

7.1.2 Eukaryotic Test Systems

7.1.2.1 Plants.

7.1.2.1.1 Yeast. Employing the yeast *Schizosaccharomyces pombe* in a host-mediated assay with B6C3F1 mice, Loprieno et al. (1979) reported findings similar to those obtained in bacteria by Henschler et al. (1977). In this study, the mutagenic potential of technical-grade TCI (composition, percent (w/w): TCI, 99; 1,2-epoxybutane, 0.19; epichlorohydrin, 0.09; ethylacetate, 0.04; n-methyl pyrrole, 0.02; diisobutylene, 0.03) was compared to a purified grade of TCI (composition w/w: TCI, 99.98 percent; chloroform, 10 ppm; carbon tetrachloride, 17 ppm; 1,1,2-trichloroethane, 21 ppm; 1,1,2,2-tetrachloroethane, 14 ppm; other contaminants, 16 ppm). One and one-half milliliters (2×10^9 cells/ml) of yeast cell suspension (SP ade 6-60/rad 10-198h$^-$) was injected into the peritoneal cavity of untreated mice (controls) or mice treated with a total volume of 0.5 ml of TCI (ranging from 0 to 2000 mg/kg) administered by gavage; cells were removed from the peritoneum and plated for counting of mutant colonies 6 or 16 hours after treatment. Each assay was performed in at least three animals. Results of this test (summarized in Table 7-2) indicated that the technical-grade material was weakly positive, showing a dose-related increase in the induction of mutants. These results are judged to be equivocal for the following reasons. The linear regression analysis presented in the report was statistically incorrect, because it was calculated using mean values rather than the individual data points. The correlation coefficient was reported to be statistically significant ($r = 0.86$); recalculating using their data, r was found to equal 0.35, which is not statistically significant. Furthermore, the number of mutants in the negative controls for the technical product was much lower, by 44 percent and 35 percent, respectively, than in the controls for the "purified" TCI and epichlorohydrin. The experimental values for the "purified" samples were in the same range as those for the technical material, and they showed no increase in mutation frequency, compared to the concurrent negative controls. The mutagenic response to epichlorohydrin tested by itself was, however, positive ($3.71 \pm 1.05 \times 10^{-4}$ mutants at 50 mg/kg), as was the response of the positive control, dimethylnitrosamine ($16.73 \pm 3.24 \times 10^{-4}$ mutants at 100 mg/kg), indicating that, for these chemicals, the assay was working properly. No data were presented on the ability of the administered TCI to reach the indicator organisms (e.g., chemical binding). The negative response in this host-mediated assay obtained for the pure sample and the equivocal response for the technical sample in this study may be due to a number of reasons, including the possibility that TCI (or its metabolites) did not reach the indicator organisms in the peritoneum under the test conditions employed.

Subsequent testing by the same laboratory (Rossi et al., 1983) was conducted to investigate further the mutagenic potential of pure and technical samples of TCI and two stabilizers contained in the technical-grade sample, epichlorohydrin and 1,2-epoxybutane. *Schizosaccharomyces pombe* strain P1 was used in *in vitro* testing and in intraperitoneal and intrasanguineous host-mediated assays. S9 mix from phenobarbital or B-napthoflavone-induced mice or rat livers was used in the *in vitro* studies in which TCI was tested at doses up to 100 mM. No increases in the frequency of forward mutations over negative controls (1.38 ± 0.67 revertants) were found. By contrast, epichlorohydrin and 1,2-epoxybutane caused dose-related increases in mutant frequency up to about a tenfold increase, compared to negative controls at 6.4 mM and 12.8 mM, respectively.

Negative responses were obtained when 2 g/kg doses of TCI were used in intraperitoneal and intrasanguineous host-mediated assays with CD-1 x C57BL mice (Rossi et al., 1983). Negative responses were also obtained when epichlorohydrin and 1,2-epoxybutane were used in intraperitoneal host-mediated assays at doses up to 2 g/kg. These data suggest that the intraperitoneal host-mediated assay in mice with *Schizosaccharomyces pombe* may not be appropriate for testing halocarbons and their stabilizers.

TABLE 7-2. MUTAGENICITY OF TRICHLOROETHYLENE AND EPICHLOROHYDRIN IN *S. POMBE* BY MEANS OF THE HOST-MEDIATED ASSAY IN B6C3F1 MICE (16 hours of incubation)

Treatment	Dose (mg/kg)	Mutants M.F. x 10^{-4} (M ± S.E.)
Trichloroethylene, technical grade	None (control)	0.94 ± 0.08*
	500	1.33 ± 0.28
	1,000	1.32 ± 0.29
	2,000	1.61 ± 0.45
Methylmethanesulfonate	50	4.16 ± 0.54
Trichloroethylene, purified grade	None (control)	1.45 ± 0.06
	1,000	1.59 ± 0.22
	2,000	1.36 ± 0.21
Dimethylnitrosamine	100	16.73 ± 3.24
Epichlorohydrin	None (control)	1.66 ± 0.03
	2	1.50 ± 1.20
	10	2.05 ± 0.51
	50	3.71 ± 1.05

*Lower than the other negative control values.

Source: Loprieno et al., 1979.

Mondino (1979) also reported negative results for purified TCI in a yeast host-mediated assay. He used *Schizosaccharomyces pombe* and adult male B6C3F1 mice. The components of the TCI sample were identified as follows: TCI, 99.9 percent; water, evaporation residue, chloroform, 1,2-dichloroethane, carbon tetrachloride, 1,1,2-trichlorethane, other impurities, all ≤20 ppm; diisopropylamine, 198 ppm; diisobutylene, 104 ppm; butylhydroxytoluene, 12 ppm. Yeast cell suspension was injected into the peritoneum of the mice, who had received 500, 1000, or 2000 mg/kg dosages of TCI by gavage. Three animals comprised each treatment group as well as the untreated control and positive control (100 mg/kg of dimethylnitrosamine) groups. After a 16-hr incubation period, the animals were sacrificed to remove yeast cells from the peritoneum for plating and counting. Plates were incubated at 32°C for 4 to 5 days before mutant colony counting. Mean numbers (± S.D.) x 10^{-4} of mutant colonies were as follows: control, 1.80 ± 0.48; low dose, 1.18 ± 1.18; mid-dose, 1.03 ±

0.91; high-dose, 1.84 ± 0.28; positive control (dimethylnitrosamine 100 mg/kg 31.1 ± 3.31. As was the case for the studies above, the negative result obtained by Mondino (1979) may result from TCI not being mutagenic, or it may be that the compound or its metabolites did not reach the test organisms in the peritoneum of the test mice.

The mutagenic properties of technical-grade TCI in the XV 185-14C haploid strain of Saccharomyces cerevisiae were assessed in vitro by Shahin and Von Borstel (1977). The ability of TCI to induce mutations at the lys 1-1, his 1-7, and hom 3-10 loci was assessed in this study. Stock cultures were incubated in liquid YPG medium at 30°C until a stationary growth phase was achieved. Cells were harvested, washed, and adjusted to a population of 1 x 10^8 cells/ml. In order to estimate its mutagenic effect in the absence of metabolic activation, cells treated with 1.11, 5.56, 111.1, or 222.2 mM of TCI during incubation at 30°C were withdrawn at 2, 6, 24, and 48 hours for plating and counting of colonies. No precautions were reported to have been taken to prevent excessive evaporation of the compound and ensure exposure to the test organism, but the reported toxicity indicates this was not a problem and that the cells were adequately exposed. Treated cells were compared with cells from similarly maintained untreated control and positive control experiments (222.2 mM of 2-acetylaminofluorene for tests without metabolic activation and 8 ug/ml of ethyl methanesulfonate for tests with and without metabolic activation). Plates were maintained at 30°C for 5 days to estimate survival, and the assays were performed in triplicate.

In the study described above, concentrations of 111.1 mM and 222.2 mM TCI, in the presence of metabolic activation, increased the reversion frequencies at the lys 1-1, hom 1-10, and his 1-7 loci (Table 7-3) but only at levels that were also highly toxic (>99 percent cell death). The revertants/10^7 survivors for lys 1-1, his 1-7, and hom 3-10, corresponding to 1-hr treatments of 111 and 222 mM, were 2552 and >23,200, 3553 and >29,000, and 5313 and >25,000, respectively, compared to negative control values of 15.3, 19.5, and 8.6. The significance of the reported increased incidences of mutations in tests conducted with metabolic activation is uncertain, because the increases were noted only when there was greater than 99 percent killing. The positive control in the tests with metabolic activation (EMS) was inappropriate because it is a direct-acting mutagen. TCI without metabolic activation was ineffective as a mutagen at all doses tested, but increased numbers of revertants were noted

TABLE 7-3. MUTAGENICITY OF TECHNICAL-GRADE TCI AT THE HIS 1-7 LOCUS OF SACCHAROMYCES CEREVISIAE

Dose (mM)	-S9 (6-hr incubation) Revertant	Percent Survival	+S9 (4-hr incubation) Revertants	Percent Survival
0	14.3	100	19.2	99
1.11	16.7	90	15.9	72
5.56	22	72	16.8	65
111.1	0	<0.13	22,000	<0.1
222.2	0	<0.013	>29,000	<0.1
Positive control	83*	61	6300†	0.23

*2-acetylaminofluorene at 20 μg/ml.

†EMS at 8 μl/ml.

Source: Adapted from Shahin and Von Borstel, 1977.

with activation. With respect to the 48-hr treatment without activation, the maximum number of revertants per 10^7 survivors for the 3 loci individually was 10 to 15 for untreated controls (98 percent survival), 12.8 to 21.4 for 1.11 mM (73 percent survival), 11.4 to 17.1 for 5.56 mM (64 percent survival), and 0 for 11.1 mM and 222.2 mM (<0.1 percent survival) of TCI. The lack of revertants with the two highest concentrations appears to correspond to failure of the cells to survive after plating. The largest number of revertants per 10^7 survivors produced by 2-acetylaminofluorene (222.2 mM) was 102 (lys 1-1) after a 6-hr treatment, and 673 (his 1-7) and 488 (hom 3-10) after a 24-hr treatment. It may be that there is a "window" of effective concentrations in which TCI is mutagenic but not excessively toxic. Such a concentration effect cannot be ruled out, because levels between 5.56 mM (ineffective) and 111.1 mM (suggestive response but highly toxic) were not tested; it was in this dose range that the weak positive responses discussed below were obtained by Bronzetti et al. (1978) and Callen et al. (1980) in strain D7. It is important to note that the composition of the TCI sample used in the study by Shahin and Von Borstel (1977) was not determined, and the possible contribution of contaminants to the response cannot be ascertained or ruled out (personal communication with R.C. Von Borstel, University of Alberta). These results do not allow a conclusive evaluation to be made.

An increase in point mutations and gene conversion by a certified ACS reagent grade of TCI in Saccharomyces cerevisiae strains D4 and D7 was observed by Bronzetti et al. (1978) in cell suspension and in host-mediated assays. The authors did not report a detailed chemical analysis of the sample; however, a description of the product obtained by personal communication with Fisher Scientific Company, the producer of the test sample, indicates a purity of >99.5 percent and stabilization with either 25 ppm diisopropylamine or 20 ppm triethylamine. In the cell-suspension experiment, strain D7 cells, which can detect mitotic gene conversion at the trp locus, point (reverse) mutation at the ilv locus, and mitotic recombination at the ade locus, were incubated in the presence of TCI with or without S9 mix derived from livers of uninduced male CD-1 mice for 4 hours at 37°C before plating on selective media (for counting of ilv revertants, ade recombinants, and trp convertants) and on complete media (for survivor counts). The results for ilv revertants are presented in Table 7-4. The other results will be discussed later, in the section on tests indicating DNA damage. In the host-mediated assay, yeast cell culture was instilled into the retroorbital sinus of male adult CD-1 mice. Two experiments were included in this assay: an acute experiment, in which a single gavage dose of 400 mg/kg was given immediately after introduction of strain D4 or D7 cells on the day of the assay. Mice were sacrificed 4 hours following introduction of the yeast cells, and liver, lungs, and kidneys were removed for homogenization and subsequent plating of yeast cells as described for the suspension assay. Assays in this study were done in triplicate or quadruplicate, and the data for gene revertants are presented in Table 7-4 as mean ± S.E.

The results of Bronzetti et al. (1978) were as follows. In the absence of metabolic activation, 10 mM and 40 mM concentrations of TCI effected a dose-related reduction in cell viability in the cell suspension system but did not induce a mutagenic response. Survival was reduced to approximately 87 percent with the 10-mM dose and to 65 percent with the 40-mM dose, compared to 100 percent for the controls. TCI did produce concentration-dependent increases in point mutation frequencies with metabolic activation at doses of 10, 20, 30, and 40 mM. Positive responses were also observed in the host-mediated

TABLE 7-4. MUTAGENICITY OF REAGENT-GRADE TCI AT THE *ILV* LOCUS IN *SACCHAROMYCES CEREVISIAE*

Suspension Tests Strain D7

Concentration (mM)	Revertants/10^6 (Mean x ± S.E.)	Percent Survival
Without Metabolic Activation		
0	0.75 ± 0.12	100
10	0.85 ± 0.15	87.5 ± 1.0
40	0.87 ± 0.06	64.1 ± 2.1
With Metabolic Activation		
0	0.85 ± 0.06	100
10	1.16 ± 0.16	81.6 ± 2.9
20	1.77 ± 0.12	67.1 ± 2.8
30	2.24 ± 0.17	55.5 ± 1.7
40	3.23 ± 0.32	50.3 ± 1.4

Host-Mediated Assay in CD-1 Mice

Strain	Organ	Treatment	No. of Mice	Revertants/10^6 x ± S.E.
Acute Exposure (400 mg/kg)				
D7	Liver	Control TCE	3 4	0.08 ± 0.04 0.56 ± 0.52
	Lungs	Control TCE	2 3	0.08 ± 0.002 0.40 ± 0.19
	Kidneys	Control TCE	2 4	0.21 ± 0.02 0.79 ± 0.37

Source: Adapted from Bronzetti et al., 1978.

assays. Fourfold to eightfold increases were obtained in the number of revertants compared to the negative controls. The highest response was observed in yeast recovered from the kidney, in which $0.79 \pm 0.37 \times 10^{-6}$ revertants were found in the treated animals versus $0.21 \pm 0.02 \times 10^{-6}$ in the negative controls.

Like Bronzetti et al. (1978), Callen et al. (1980) also employed a TCI sample obtained from the Fisher Scientific Company. They used *Saccharomyces cerevisiae* strains D4 and D7 to assess TCI's genetic activity and strain D5 intact yeast cells or microsomal suspensions to study its metabolism indirectly. Gene conversion at the *trp 5* locus, mitotic recombination at the *ade 2* locus, and gene reversion of the *ilv 1* locus (Table 7-5) of strain D7 were assessed by exposures of the cells to 0, 15, and 22 mM solutions in suspension at 37°C for 1 hour followed by plating on appropriate media, allowing for expression of genetic activity and, finally, scoring. A twofold increase in reverse mutations at the *ilv 1* locus over the spontaneous control values was observed at 15 mM (7.4 vs. 3.6 revertants $\times 10^{-6}$ survivors, respectively) without an exogenously supplied metabolic activation system (67 percent cell survival). A severely toxic effect was observed at 22 mM. A positive response without activation was observed by Callen et al. (1980) but not by Bronzetti et al. (1978). The reason for this is not known, but it may be due to the growth stage of the yeast cells when tested. Callen et al. (1980) found TCI to be mutagenic to cells from log phase cultures but not to cells from stationary cultures of D7. Bronzetti et al. (1978) did not report the growth phase of the cells used in their test, but if they employed stationary phase cells, a negative response without activation would be expected.

Callen et al. (1980) provided additional suggestive evidence that *Saccharomyces* can metabolize TCI. They analyzed whole-cell suspensions of strain D5 in the log phase of growth in a spectrophotometer, after exposure to unspecified amounts of TCI. Peaks with maximums of 450 and 505 mM were observed. The peak at 450 nm appeared to reach a maximum after roughly 5 minutes, and after this period an additional peak at 423 nm was observed. This spectrum is similar to that observed when TCI is added to mammalian microsomes. These results indicate that TCI enters exposed yeast cells and suggest TCI is metabolized by a yeast cytochrome P_{450}-independent monooxygenase system.

TABLE 7-5. MUTAGENICITY OF TCI AT THE *ILV* LOCUS IN
SACCHAROMYCES CEREVISIAE D7

	Survival		*ilv* 1	
Concentration (mM)	Total Colonies	Percent	Total Revertants	Revertants 10^6 Survivors
0	1291	199	43	3.6
15	802	67	59	7.4
22	3	0.3		

Source: Adapted from Callen et al., 1980.

The yeast studies show that commercially available TCI is mutagenic in *Saccharomyces cerevisiae*. The data are consistent with TCI metabolites being mutagenic, because the samples tested are represented as being free from epoxide stabilizers (which are direct-acting mutagens), and metabolic activation appears to be required for the genetic activity of TCI. The doses required to elicit twofold to fourfold increases in gene reversion (i.e., 10 to 40 mM in suspension tests and 400 mg/kg in the host-mediated assay) show the commercial samples of TCI to be weakly active. The negative responses obtained with the *Schizosaccharomyces pombe* host-mediated assays are not considered to diminish the positive results in *Saccharomyces* because of the inherent insensitivity of the *S. pombe* test (forward mutation assay with high spontaneous control levels and no selection scheme).

7.1.2.1.2 *Angiosperms*. Testing for somatic cell mutations in the stamen hairs of *Tradescantia* clone 4430, an interspecific hybrid (T. *subacaulis* x T. *hirsutiflora*), was done by Schairer et al. (1978) to assess the mutagenic potential of TCI. A group of unrooted fresh cuttings containing young inflorescences with flower buds in a range of developmental stages was exposed to 0.5 ppm TCI for 6 hours. Following treatment, the cuttings were grown in aerated Hoagland's nutrient solution and analyzed each day as they bloomed for 3 weeks after treatment. One hundred forty-eight mutations were observed out of 44,000 stamen hairs scored. The mutation frequency (± S.E./100 cells), after the negative control values (not reported) were subtracted, was reported to be positive (0.112 ± 0.036; $P < 0.01$). Based on the data given, however, this value cannot be verified. The mutation frequencies (± S.E./100 cells) for 6-hr exposures to 5 ppm EMS and 75 ppm vinyl chloride were 1.012 ± 0.133 and 0.112 ± 0.046, respectively. Because the purity and source of the sample were not provided, it is not possible to ascribe with certainty the observed positive effects to the genotoxic action of TCI.

7.1.2.2 Animals.

7.1.2.2.1 Insects (*Drosophila*). Abrahamson and Valencia (1980) fed 1000 ppm (5380 mg/m^3) TCE in 0.2 percent sucrose for 72 hours to male Canton-S (wild type) *Drosophila melanogaster*, and subsequently mated 102 of these flies with female FM6 (a marked and balanced X-chromosome) flies, screening the F_2 descendents for mutagenicity using the sex-linked recessive lethal test. Ninety-six of the treated males were fertile. Administration of the test compound was performed in a glass shell vial containing filter material saturated with the feeding solution, which was renewed twice daily. However, because of the volatility of TCI, the actual dose given to the flies might have been much less than expected in the feeding study. Treated and untreated control males were mated individually with 3 females each and then transferred without anesthetization to fresh females at 2- to 3-day intervals, until a total of 4 broods of progeny were produced. The fertile females were left in the vials for 1 week. In a simultaneous experiment, 0.3 µl of TCI was injected into the abdomen of the male flies before mating. A technical-grade sample from Allied Chemical Co. was used, and the purity and composition of the sample were not ascertained (personal communications with L.Valcovic, Food and Drug Administration, sponsor of the study; and Allied Chemical Co.).

TCI was not mutagenic in the above test. The frequency of lethals (number of lethals/number of chromosomes tested) was 0.12 percent (19/9248) in the feeding experiment, 0.17 percent (4/2344) in the injection experiment, 0.31 percent (31/10,009) in concurrent controls, and 0.24 percent (230/94,491) in the historical controls used in the testing laboratory for 2 years prior to completion of this study. The negative result obtained in this test indicates that either the test substance did not reach the target site (testes) or that TCI is not mutagenic.

Beliles et al. (1980) also tested TCI in *Drosophila* for its ability to cause sex-linked recessive lethals and, in addition, for its ability to cause X or Y chromosome loss. Threehundred one-day-old $B^SY\ y^+ac^+$, $(K^S1+2)^-/X.Y^S$, INEN2,f males were placed in each of three screen cages and subsequently exposed to 0, 100, and 500 ppm (0, 538, and 2690 mg/m^3) TCI vapor from North Strong (estimated to be 99.9 percent pure by infrared spectroscopy) for 7 hours. An unspecified number of males serving as positive controls were fed 0.025 M EMS in 1 percent sucrose for 8 hours. Two hundred males per treatment group were individually mated to sequential groups of 3 In(1)sc^8, sc^8 1 B/In(1)dl-49 B^{M1}, y ac v B^{M1} virgin females. The brooding scheme consisted of a 2-3-3-2 day mating sequence, enabling specific sperm cell stages to be tested (i.e., spermatozoa, spermatid, spermatocyte, and spermatogonia). Losses of the entire X or Y chromosome and the short or long arm of the Y chromosome were detected by scoring for specific phenotypic classes in the F$_1$ generation. F$_1$ males of the genotype y ac v B^{M1}/In(1)EN2,f were mated to virgin Y^S/X.Y^L, In(1)dl-49, v f B females, and the occurrence of recessive lethal mutation on the In(1)EN2, f chromosome was detected by the lack of males with forked bristles in the F$_2$ generation. The frequencies of X or Y chromosome loss were 0.07 percent and 0.17 percent for the low and high doses, respectively, compared to 0.06 percent for the concurrent negative control. These values are not significantly different; however, the 0.17 percent was close to a significant value. The positive control EMS did not induce sex chromosome loss under these test conditions; thus, it is not possible to determine whether the test was conducted properly. The frequencies of losses of the long arm of the Y chromosome, 0.03 percent and 0.05 percent for the low and high doses, respectively, and losses of the short arm of the Y chromosome, 0.02 percent for both dose levels, were not significantly different from the 0.01 percent value of the concurrent negative control for losses of both arms. Like the study by Abrahamson and Valencia (1980), Beliles et al. (1980) found TCI to be ineffective in causing an increase in the incidence of sex-linked recessive lethal mutations. The frequencies were 0.03 percent and 0.16 percent for the low and high doses, respectively, compared to 0 percent for the concurrent negative control and 0.11 percent of the historical negative control. The frequency of sex-linked recessive lethal mutations for the positive control (EMS) was 21.56 percent showing a significant positive response. No significant variations were observed among the four germ cell (brood) stages employed in any of the assays.

7.1.2.2.2 Rodents. TCI was tested in a mouse spot test by Fahrig (1977). Virgin females of the inbred C57BL/6J Han strain (a/a, wild type) were mated with males of the Han-rotation bred T-stock (a/a=non-agouti, a/a=brown, c^{ch}

p/c^{ch} p=chinchilla and pink-eyed dilution; d se/d se=dilute and short ear; s/s=piebald spotting) to yield embryos of the genotype a/a, b/+, $c^{ch}p/+$ +, d se/+ +, s/+ (black coat, dark eyes). Each male was mated with two females. On day 11 of gestation, a single intraperitoneal (i.p.) injection of TCI (99.5 percent pure) was made into 50 and 26 females at doses of 140 and 350 mg/kg, respectively. Impurities detected in this test sample were 60 to 150 ppm ethanolamine, 0.0003 percent chloride (Cl_2), and 0.0001 percent free chloride (Cl-1) (personal communication with R. Fahrig, University Tubingen, West Germany). Litters were born on day 21 of gestation, and pups were examined for color spots, the mutagenic endpoint, twice weekly between 2 and 5 weeks of age.

The number of offspring (number of litters) surviving for examination was 144 (23) in the untreated control group, 145 (38) in the low-dose group, and 51 (13) in the high-dose group. The number of coat color (brown or gray) spots found were none in concurrent control pups, 2/794 historical control pups (0.25 percent), 2 low-dose pups (1.3 percent), and 2 high-dose pups (3.92 percent). Known mutagens, including ethyl methanesulfonate, were positive (26 percent at 0.9 mM EMS). Results of this study indicate that the TCI sample tested or one or more of its metabolites is able to produce mutations in somatic tissue of an intact mammal.

Based on the weak positive responses obtained in *Saccharomyces*, *Tradescantia*, and mice, commercial samples of TCI are judged to be capable of causing gene mutations after metabolic activation. Because of the requirement of activation, it is not likely that epoxide stabilizers present in the commercial samples account for the effect. However, because high doses of commercial-grade TCI were required to elicit a positive effect, the material must be considered to be only weakly mutagenic. The data for purified samples of TCI do not conclusively show it to be positive. However, weak, less than twofold increases are reported with metabolic activation at high doses.

7.2 CHROMOSOME ABERRATION STUDIES

TCI has been examined for its ability to cause changes in chromosome number (chromosomal loss tests with *Drosophila*, micronucleus test in mice, and hypodiploid cells in humans), and structure (in studies employing mice, rats, and humans). The results of the chromosome loss test were described in the previous section (Beliles et al., 1980). The rest of the studies are presented next.

7.2.1 Experimental Animals

7.2.1.1 Bone Marrow. Beliles et al. (1980) tested TCI for its ability to cause chromosome aberrations in rat bone marrow cells. Adult male and female rats [CRL: COBS CD (SD) BR] were exposed to 0, 100, or 500 ppm (0, 538, or 2690 mg/m^3) TCI by inhalation, both acutely and subchronically. The positive control was TEM at 0.3 mg/kg given i.p. in 0.85 percent saline. Acute exposure consisted of a single 7-hr session in the exposure chamber. Subchronic exposures consisted of 5 daily exposures of 7 hours. Bone marrow cells were then collected; slides were prepared and coded, and 50 cells/animal were scored. There was no increase in the number of aberrations in either study. The percentage of cells with aberrations were 0.4 to 2.4 in the negative control group compared to 0 to 2.7 in the TCI-treated group. The positive control TEM was clastogenic, causing 7.2 to 8.5 percent aberrant cells. TCI failed to cause the induction of aberrations or aneuploidy under the conditions of this study; however, it is not considered to be an adequate test of the clastogenic potential of TCI. The positive control was not appropriate, because it was given by i.p. injection and TCI was administered via inhalation. In addition, the doses used may have been too low to detect weak activity. The lowest concentration in air reported to cause death in the rat is 8000 ppm/4 hour (American Industrial Hygiene Association, 1969). It would have been appropriate to have tested higher doses of TCI and to have incorporated a volatile and preferably a chemically related substance as the positive control.

7.2.1.2 Dominant Lethal Test. The precise nature of damage-causing dominant lethal effects is not known, but there is a good correlation between chromosome breakage in germ cells and dominant lethal effects (Matter and Jaeger, 1975). When dominant lethal effects are observed, it can be concluded that the test substance reaches the gonads and likely causes genetic damage.

Slacik-Erben et al. (1980) assessed the ability of 0, 50, 202, and 450 ppm (0, 269, 1086, and 2421 mg/m^3) TCI in air to cause dominant lethal mutations in NMRI-Han/BGA mice. The sample contained 99.5 percent TCI stabilized with 100 mg/l triethanolamine. Analysis of a liquid sample also identified 5 ppm 1,2-dichloroethylene, 9 ppm cis-1,2-dichloroethylene, tetrachloride, 10 ppm 1,1,2-trichloroethane, and 815 ppm tetrachloroethylene. Fifty males per dose were exposed for 24 hr in a 1000-l volume glass chamber equipped with a ventilator. The temperature was kept between 22-24°C and the humidity between 70-80 percent. For rapid equilibration the calculated volume of liquid TCI was placed in the chamber, and mixtures of TCI and air were streamed through the chamber at a rate of 200 (50 and 202 ppm) and 300 (450 ppm) l/hr. After the 24-hr exposure period, the male mice were mated individually with one untreated NMRI-Han female for days. At this time, the mated females were replaced by fresh virgin females who were kept for 4 days and then replaced (and this was repeated, etc.), until each male had been mated singly to 12 females for 4 days each (up to 48 days after treatment). By this mating regimen, all stages of spermatogenesis were sampled, from mature sperm to gonial cells. Each pregnant female was sacrificed by cervical dislocation, dissected, and examined for dead and living implants. The results of the testing are presented in Table 7-6. Under conditions of this test, TCI was not detected as mutagenic, but there are several important points to consider with respect to this finding. The males exhibited no visible effects during the exposure, and doses up to 450 (2421 mg/m^3) ppm did not alter the fertilization rate. Thus, the doses selected for test may not have approached the maximum tolerated dose. Even if the maximum tolerated dose was approached, the dominant lethal test is not recognized to be a sensitive test for detecting mutagens (Russell and Matter, 1980), due to the high spontaneous level of dominant lethal events.

7.2.1.3 Micronucleus Test. Duprat and Gradiski (1980) used TCI in a micronucleus test. CD-1 mice were divided into 9 groups, 6 of which were given up to 3000 mg/kg TCI (analytical grade 99.5 percent purity; unspecified source) in gum arabic orally in two dosings, separated by 24 hours. The animals were sacrificed 16 hours later; bone marrow smears were made and stained, and polychromatic erythocytes (PCEs) and mature erythrocytes (MEs) were examined for the presence of micronuclei. The incidence of micronuclei in PCEs was higher in the vehicle control group (i.e., treated with 10 percent gum arabic solution) than in the untreated control (1.0 ± 0.5 versus 0.5 ± 0.4 percent). Also, the incidence of what were considered micronuclei in PCEs and MEs from treated animals was greater than the vehicle control levels at all doses and showed a dose-response effect. At the highest dose (3000 mg/kg) the percentage of PCEs with "micronuclei" was 15.8 ± 5.7 compared to the vehicle and untreated control values presented above. The corresponding values for "micronuclei" in MEs were 3.4 ± 1.8, 0.2 ± 0.3, and 0.3 ± 0.3, respectively ($P < 0.01$). Because of their occurrence in MC, the micronuclei observed by Duprat and Gradiski (1980) are likely artifactual (see discussion by Schmid, 1975).

TABLE 7-6. MUTAGENICITY OF TCI IN THE DOMINANT LETHAL ASSAY, USING NMRI MICE

Dose in ppm (rate)	Female		Total Implants	Total Dead Implants	Total Live Implants	Live Implants/ Female	% Dominant Lethal Factors*
	Mated	% w/Implants					
(200 1/h)							
0	498	89.6	5578	443	5135	11.5	
50	482	90.1	5360	479	4881	11.2	2.6
202	493	91	5703	531	5172	11.5	0
(300 1/h)							
0	503	93.4	6142	502	5640	12.0	
450	488	92.4	5927	533	5394	11.97	0.25

*Dominant lethal factors (F_L).

$$F_L = 1 - \frac{\text{(live implants per female of test group)}}{\text{(live implants per female of the control group)}} \times 100$$

Source: Adapted from Slacik-Erben et al., 1980.

7.2.2 Humans

Konietzko et al. (1978) studied the incidence of chromosome aberrations (structural and numerical) in peripheral lymphomycytes of 28 chemical workers engaged in the manufacture of TCI. The workers ranged in age between 23 and 67 years, with an average age of 42.5. Their exposures ranged in duration from 1 to 21 years; the average length of exposure was approximately 6 years. Since many of the workers had been exposed over a period of many years, it was not known to what other chemical substances they may have been exposed. Thus, the study could only address the long-term effects associated with working in a factory that manufactured TCI. A control group of 10 healthy young men working in the Institute of Human Genetics, where the study was conducted, and ranging in age from 15 to 45 years (average age 28.3 years), was selected for comparison. However, this group was not a matched control group. The exposed group had a frequency of 10.96 ± 4.4 percent (X ± SD) hypodiploid cells, which was significantly elevated compared to the control group, 6.5 ± 3.2 percent hypodiploid cells. Concerning chromosome breaks/100 cells, the frequencies were 3.1 ± 3.6 and 0.6 ± 0.69, respectively. Because the control group was not a matched control group and the mean frequency of chromosome breaks observed in the exposed workers was within the normal range, the increased incidence of chromosome breaks was not considered significant by the authors, although they did consider the increase in hypodiploid cells significant. Because of this, it was thought appropriate to further investigate factors which may have contributed to the increased incidence of hypodiploid cells. Based on the frequency of hypodiploid cells in the controls, it was calculated that individuals possessing >13 percent hypodiploid cells were abnormal. Workers were divided according to whether or not they possessed >13 percent hypodiploid cells, and these groups of workers were subsequently further characterized (Table 7-7). Nine individuals had greater than 13 percent hypodiploid cells. These individuals worked under higher daily peak concentrations of TCI (as measured on three occasions over a 2-week period for 8 hr each time) than did their fellow workers. The average daily concentrations for these 2 groups of workers were 75 ± 14 ppm versus 61 ± 23 ppm, respectively. These exposure levels were used as estimates of previous years of exposure. This was thought to be a good approximation of the actual exposures received, because no technical changes had been made in the plants. This report may provide suggestive evidence that the exposures resulting from the manufacture of technical-grade

TABLE 7-7. NUMERICAL CHROMOSOME ABERRATIONS IN TCI WORKERS

Parameter	>13% Hypodiploid Cells (n* = 9) Mean Value (X)	Standard Error (S_x)	>13% Hypodipoloid Cells (n* = 18) Mean Value (X)	Standard Error (S_x)
Daily average concentration (ppm)	75.0	17.4	61.4	23.1
Average maximum concentration (ppm)	205.8	96.3	115.7	57.6
Exposure (years)	5.9	4.8	5.9	5.1
Total dose (ppm x day)	0.55×10^6	0.72×10^6	0.31×10^6	0.39×10^6
Age (years)	38	9.4	40.4	9.7
Alcohol (g/day)	18.9	29.3	33.6	37.5
Nicotine (cigarettes/day)	12.8	12.7	10.1	10.2

*n = Number of individuals studied.

Source: Adpated from Konietzko et al., 1978.

TCI may cause chromosomal loss or nondisjunction in humans. However, the lack of an appropriate matched control group and the possibility that the incidence of hypodiploid cells was due to preparation of the chromosomes rather than to exposure to TCI renders this study inconclusive.

7.3 OTHER STUDIES INDICATIVE OF MUTAGENIC ACTIVITY

Additional studies have been conducted on the genotoxicity of TCI. These studies do not measure mutagenic events per se, in that they do not demonstrate the induction of heritable genetic alterations, but positive results in these test systems show that DNA has been affected. Such test systems provide supporting evidence useful for qualitatively assessing genetic risk.

7.3.1. Gene Conversion in Yeast

An increase in the frequency of mitotic gene conversion has been observed after exposure to chemical mutagens as a consequence of genetic repair (Fabre and Roman, 1977). Bronzetti et al. (1978) tested TCI for its ability to induce this end point at the *ade* and *trp* loci in strains D4 and D7 of *Saccharomyces cerevisiae*. Strain D7 cells were incubated with TCI (ranging up to 40 mM) with or without CD-1 mouse liver S9 mix for 4 hours at 37°C in a liquid suspension assay. A dose-related increase in trp^+ convertants was observed in the tests conducted with metabolic activation ($41.0 \pm 2.6 \times 10^{-6}$ convertants at 40 mM compared to $16.3 \pm 0.42 \times 10^{-6}$ convertants for the negative control). No increase was noted in the test conducted without activation. Toxicity was observed in both tests with a 40 to 50 percent reduction in cell survival at the highest dose (40 mM). Twofold to fivefold increases in the incidences of gene conversion were noted in these tests and also in the host-mediated assays where strains D4 and D7 were instilled into the retroorbital sinus of adult male CD-1 mice. TCI was given by gavage in an acute-exposure experiment (single dose of 400 mg/kg) or a subacute-exposure experiment (22 pretreatment dosages of 150 mg/kg 5 days/week followed by a single 400 mg/kg dose). The largest increases (fourfold over spontaneous values) were found in yeast isolated from the liver and kidney (target organs for TCI toxicity), and gene conversion frequencies were higher with subacute dosing, compared to acute dosing.

Callen et al. (1980) treated D7 cells to 0, 15, and 22 mM solutions of TCI in liquid suspension tests at 37°C for 1 hour followed by plating on selective media. Cell survival was reduced by 30 percent at 15 mM and by >99 percent at 22 mM TCI. A fivefold increase in trp convertants was detected at 15 mM compared to the negative controls (76×10^{-6} versus 14×10^{-6}, respectively). There was also a fourfold increase in mitotic recombination at the ade 2 locus at 15 mM TCI compared to the negative controls (12×10^{-3} vs. 3×10^{-3}, respectively). These end points were not reported for the cultures exposed to 22 mM TCI because of the extreme toxicity at this dose. The positive responses for gene conversion and mitotic recombination in Saccharomyces strain D7 indicate TCI is capable of interacting with DNA in yeast.

7.3.2. Sister Chromatid Exchange (SCE) Formation

White et al. (1979) evaluated the ability of anesthetic-grade TCI (source and purity not reported) to cause sister chromatid exchanges (SCE) in Chinese hamster ovary cells. The material was administered as a gas to exponentially growing cells at 0.17 percent of the atmosphere in the presence of 19 percent oxygen, 5 percent carbon dioxide, and 75.83 percent nitrogen for 1 hour with S9 mix prepared from Aroclor 1254-treated male rats. After exposure, the concentration of TCI gas in the flask was measured by gas chromatography and found to be 59 percent of that delivered. The cells were allowed to go two rounds of cell division in the presence of bromodeoxyuridine (BrdU). Subsequently, chromosome preparations were made and stained with Giemsa. Onehundred control and 100 treated metaphases were scored for SCEs by a single observer. The number of SCEs/chromosome were 0.536 ± 0.018 and 0.514 ± 0.018, respectively. Under the conditions of this test, anesthetic-grade TCI did not yield an increased incidence of SCEs. However, it should be noted that the conditions of the test may not have provided an adequate assessment of TCI's ability to cause sister chromatid exchanges. In the first place, only one dose was administered, and it may have been too low. There was no indication of toxicity in the treated cells, further suggesting that they did not receive an adequate dose. In the second place, no concurrent positive controls were employed to ensure that the test system was working properly. These deficiencies reduce the weight of the negative response reported by White et al. (1979).

Gu et al. (1981) collected blood from six individuals occupationally exposed to TCI, cultured their peripheral lymphocytes, and scored the chromosomes for SCEs. Blood was also collected from nine additional persons who served as the control group and as blood donors for testing trichloroethanol and chloral hydrate, metabolites of TCI, *in vitro* for the induction of SCEs. A significantly higher number of SCEs/cell was reported for the exposed group compared to the controls (9.1 ± 0.4 versus 7.9 ± 0.2). There was no information about the levels of TCI to which the subjects were exposed in the workplace, but there was a good correlation between the average number of SCEs/cell per person and the levels of trichloroacetic acid and trichloroethanol measured in the blood. Those with the highest mean value for SCEs also had the highest level of these metabolites of TCI in their blood. Cultured lymphocytes were exposed to trichloroethanol and chloral hydrate at 178 and 54.1 mg/l, respectively, for 68 to 72 hours, and increases in SCEs were observed, compared to the untreated cultures. The results are tabulated as follows:

Group	No. of Karyotypes	X ± SD	Comparison Between	P Value from t Test*
1. Control	212	7.9 ± 3.0		
2. Trichloroethanol (1)	24	9.8 ± 3.2	1 & 2	<0.05
3. Trichloroethanol (2)	72	9.2 ± 3.3	1 & 3	<0.01
4. Chloral hydrate	52	10.7 ± 4.0	1 & 4	<0.001
5. TCI *in vivo*	194	9.1 ± 4.9	1 & 5	<0.01

*Calculated by Gu et al.

The results obtained by Gu et al. (1981) are suggestive of a positive response. The chemical substances to which the TCI workers in this study (Group 5) were exposed and the extent to which such exposures occurred are unknown. It is likely that they were exposed to other substances which have been shown to be mutagenic (e.g., 1,2-epoxybutane and epichlorohydrin), and these may be responsible for the weak effects. However, the suggestive responses obtained by Gu et al. (1981) in *in vitro* studies with trichloroethanol (>99.5 percent pure, Fluka; Groups 2 and 3) and crystalline chloral hydrate (Group 4) show that these metabolites of TCI could be responsible for the induction of SCEs in TCI workers.

7.3.3. Unscheduled DNA Synthesis (UDS)

Four tests have been performed to assess the ability of TCI to cause unscheduled DNA synthesis (Beliles et al., 1980; Perocco and Prodi, 1981; Williams and Shimada, 1983; Williams 1983). Although demonstration that a chemical causes unscheduled DNA synthesis does not provide a measurement of its mutagenicity, it does indicate that the material interacts with DNA.

Beliles et al. (1980) exposed human WI-38 cells to TCI at doses ranging from 0.1 to 5.0 µl/ml to evaluate its ability to cause UDS. The positive controls N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and benz(a)pyrene (BaP) were used at concentration ranges between 1.25 to 10 µg/ml and 2.5 to 20 µg/ml, respectively. Both agents were positive; however, BaP produced a non-linear dose-response effect. All studies were conducted at the same time on a single batch of WI-38 cells. TCI yielded a weak increase at the two lowest doses (1.5- to 1.8-fold increase), both with and without activation (Table 7-8). The response is only suggestive of an effect, but the testing done with S9 may not have provided an optimal test of its genotoxic potential, because the positive control (BaP) was only effective at one dose (2.5 µg/ml); higher doses resulted in a negative response, although this may have been due to cell killing.

Perocco and Prodi (1981) collected blood samples from healthy humans, separated the lymphocytes, and cultured 5×10^5 of them in 0.2-ml medium for 4 hours at 37°C in the presence or absence of TCI (Carlo Erban, Milan, Italy or MerckSchuchardt, Darmstadt, FRG, 97-99 percent pure). The tests were conducted both in the presence and in the absence of PCB-induced rat liver S9 mix. A comparison was made between treated and untreated cells for scheduled DNA synthesis (i.e., DNA replication) and unscheduled DNA synthesis. A difference was noted between the groups, with respect to scheduled DNA synthesis measured as dpm of [^{3}H] deoxythymidylic acid (TdR) after 4 hours of culture (2661 ± 57 dpm in untreated cells, compared to 1261 ± 36 dpm in cells treated with 5 µl/ml TCI). Subsequently, 2.5, 5, and 10 µl/ml (0.028, 0.056, and 0.11 µmole/ml) TCI was added to cells which were cultured in 10 mM hydroxyurea to suppress scheduled DNA synthesis. The amount of unscheduled DNA synthesis was

TABLE 7-8. UNSCHEDULED DNA SYNTHESIS IN WI-38 CELLS

Test	Compound Concentration (µl/ml)	DNA (µg)	DPM	DPM/ (µg DNA)	Percent of Control	Response
Nonactivation						
Solvent control, DMSO	0	23.76	988.7	41.6	100	
MNNG	5 µg/ml	6.27	472.9	75.4	181	+
TCI	0.1	24.75	1561.3	63.1	152	+
	0.5	23.76	1616.1	68.0	164	+
	1.0	26.07	1221.3	46.8	113	-
	5.0	13.20	150.6	11.4	27	-
Activation						
Solvent control, DMSO	0	24.75	834.5	33.7	100	
BaP[b]	2.5 µg/ml				170	+
TCI	0.1	29.70	1500.3	50.5	100	-
	0.5	28.38	1622.1	57.2	170	+
	1.0	21.78	860.6	39.5	117	-
	5.0	21.78	1006.8	46.2	137	-

Source: Adapted from Beliles et al., 1980.

estimated by measuring dpm from incorporated [^{3}H]TdR 4 hours later. At 10 µl/ml TCI, 392 ± 22 and 439 ± 23 dpm were counted, without and with exogenous metabolic activation, respectively. Both values were lower than corresponding negative controls of 715 ± 24 and 612 ± 26 dpm, respectively. No positive controls were run to ensure that the system was working properly, although testing of chloromethyl methyl ether (CMME) with activation resulted in a doubling of dpms over the corresponding negative control values (1320 ± 57 at 5 µl/ml CMME versus 612 ± untreated). The authors calculated an effective DNA repair value (r) for each chemical, based on the control and experimental values with and without metabolic activation. TCI was evaluated by the authors as positive in the test, but they did not state their criteria for classifying a chemical as positive. It is important to note that none of the experimental values from cells treated with TCI without metabolic activation had higher dpm values than the controls. Furthermore, only one out of three experimental values was greater than the controls with metabolic activation (668 ± 34 at 5 µl/ml, compared to control value of 612 ± 26). The increase was not statistically significant. Thus, the positive finding reported in this work is judged to be inconclusive.

In unpublished papers, Williams and Shimada (1983) and Williams (1983) tested samples of TCI for their ability to cause unscheduled DNA synthesis in rat and mouse primary hepatocyte cultures (HPC DNA repair assay). Williams and Shimada (1983) report two samples were tested using rat primary hepatocytes. One was fully stabilized, and the other, referred to as "non-stabilized," contained 99.9 percent TCI. No information was provided about the remaining components. Two types of tests were conducted. One was a conventional HPC DNA repair assay in that the hepatocytes were exposed to TCI added to the culture medium. The other was a modified assay, and the cells were placed in small glass dishes in a sealed incubator and exposed to TCI vapor. Tritiated thymidine (^{3}H-TdR) was added to the culture medium in both types of tests. If TCI caused DNA damage repaired by the excision repair pathway, ^{3}H-TdR would be incorporated into the repaired DNA. To detect such incorporation, the cells were fixed on glass slides and subsequently coated with radiotrack emulsion. After an incubation period, the slides were developed and examined microscopically to count developed silver grains in the radiotrack emulsion over the cell nuclei.

Williams and Shimada (1983) used 2-acetylaminofluorene and vinyl chloride as positive controls for the conventional and modified exposure assays, respectively. 2-acetylaminofluorene was highly active (170 grains/nucleus at 10^{-5}M) and by comparison, vinyl chloride was weakly active at 2.5 percent (5 grains/nucleus) and 5 percent (11 grains/nucleus) in increasing unscheduled DNA synthesis compared to the negative controls (3 grains/nucleus).

In the modified HPC DNA repair assay, cells were exposed to dosages up to 2.5 percent TCI in the air for 3 or 18 hours. No increase in the grain count was observed (the number of grains/nucleus ranged from 0.2 ± 0.2 to 3.2 ± 2.5 in treated cells, compared to 2.75 ± 2.76 in the untreated controls). Similarly, negative results were reported in the conventional assay, in which the cells were exposed to concentrations as high as 1 percent TCI in the medium.

There are several factors to consider in the analysis of this assay. First, the system is designed to detect the occurrence of a specific type of repair (i.e., "long-patch" excision repair). Xenobiotics which cause damage that is not repaired or that is repaired by another mechanism will not be detected as possessing genotoxic activity in the system.

Second, the end point measured may not actually reflect repair synthesis of chromosomal DNA in the nucleus (Lonati-Galligani et al., 1983). Enhanced incorporation of ^{3}H-TdR into mitochondrial DNA or suppressed ^{3}HTdR incorporation into mitochondrial DNA, without a concomitant increased or decreased incorporation into nuclear DNA, can lead to false negative or positive results, respectively. Thus, both nuclear incorporation and cytoplasmic incorporation should be measured and the dose-responses plotted for each to serve as a basis for deciding whether or not the compound has induced UDS.

Third, nuclear grain counts vary with nuclear size (Lonati-Galligani et al., 1983); thus, it may be appropriate to express grain counts as a percentage of the measured nuclear area. In the studies by Williams and Shimada (1983) the values were transformed by subtracting cytoplasmic grain counts from total nuclear grains counts to give a net grain count; as a result, the data are difficult to evaluate, and the way they are presented may preclude the detection of weakly active agents. Thus, although the results of this study are negative, they do not provide convincing evidence that TCI does not induce UDS. This is a relevant point, because vinyl chloride, a structurally related compound and known mutagen, was only weakly active in this test and then only at doses as high or higher than the highest dose tested for TCI.

In the Williams (1983) study, TCI (source and purity not reported) was assayed in the HPC DNA repair test using a conventional liquid exposure protocol. Primary hepatocytes from B6C3F1 mice and Osborne-Mendel rats were tested in two separate test series. A positive dose-related response was obtained in the mouse cells between 10^{-4}M and 10^{-2}M (18.24 ± 3.95 grains/nucleus at 10^{-2}M vs. 0.78 ± 1.18 grains/nucleus in the concurrent negative control). TCI was reported to be nontoxic between 10^{-6}M and 10^{-2}M. A negative response was obtained in a test conducted in rat cells. Positive and negative controls were employed for both sets of experiments and responded appropriately, but the aforementioned concerns regarding the Williams and Shimada (1983) study apply here as well. In spite of this, the positive response in the mouse cells shows that a commercially available sample of TCI is genotoxic in B6C3F1 mouse liver cells.

The positive responses for gene conversion and mitotic recombination in yeast, and UDS in mouse cells, provide evidence that commercially available TCI is weakly active in damaging DNA.

7.4 EVIDENCE THAT TCI REACHES THE GONADS

Although not bearing on the ability of TCI to cause mutations, two studies have been conducted to assess its ability to induce sperm morphological abnormalities in mice (Land et al., 1979; Beliles et al., 1980). A statistically significant increase was observed in both studies, strongly suggesting that the compound or an active metabolite reaches the gonads. Land et al. (1979) exposed groups of (C57BL/C3H)F_1 male mice (13 weeks old) to air (negative control), 0.02 percent and 0.2 percent anesthetic-grade TCI vapor 4 hr/day for 5 consecutive days. Twenty-eight days after the first day of exposure the animals were sacrificed. Both cauda epididymides were removed, minced, strained, and stained in 1 percent Eosin Y. Slides were prepared, mounted, coded, and evaluated by scoring 1000 sperm/slide at 400 X magnification. Data were recorded as percent abnormal sperm. The means were calculated for each group and compared with controls using the sample t-test. As noted below, TCI exposure resulted in statistically significant elevations in the percentage of morphologically abnormal sperm.

Agent	Concentration %	Number of Animals Studied	% Abnormal Sperm ± S.E.
Air		15	1.42 ± 0.08
TCI	0.02	5	1.68 ± 0.17
	0.2	5	2.58 ± 0.29*

*Statistically significant difference compared to controls (P <0.001).

This study provides suggestive evidence that exposure to a high concentration of TCI during the period of meiotic division damages early spermatocytes.

Beliles et al. (1980) also observed a significant increase in sperm head abnormalities in male CD-1 mice, but not albino rats [CRL: COBS CD(SD) BR]. Groups of 12 animals were dosed 7 hr/day for 5 days to 0, 100, or 500 ppm (0,538, or 2690 mg/m^3) TCI. After dosing, groups of 4 animals were killed at the end of 1 week, 4 weeks, and 10 weeks. Sperm were collected from the caudae epididymides and suspended in 0.85 percent saline, stained, and scored for abnormalities. At least 2000 sperm were analyzed/group (500/animal). The negative responses obtained in the rat study are not considered adequate for assessing the ability of TCI to reach the testes, because the positive controls gave a negative response. Increased incidences of abnormal sperm were observed in the mice of the 500 ppm (2690 mg/m^3) group compared to the negative controls for weeks 1 and 4 (i.e., 18.9 percent versus 6.8 percent and 23.5 percent versus 8.1 percent, respectively). A significant increase was also noted in the 100-ppm exposure group, compared to the negative control for week 4 (14.8 percent versus 8.1 percent).

7.5 MUTAGENICITY OF METABOLITES

Trichloroethylene is metabolized to trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid. There are several intermediates leading to the formation of these end products of metabolism, including some which have been identified in man (e.g., chloral hydrate) and some which have not been identified but which are thought to occur (e.g., TCI oxide). Some mutagenicity studies bearing on a few of TCI's metabolites are discussed below. See the chapter on metabolism for a more detailed discussion.

Kline et al. (1982) tested the mutagenic potential of TCI oxide in Salmonella strain TA1535 and E. coli strain WP2 uvrA. They observed no increases in revertant colonies at doses ranging from 0.25 to 5 mM. Preincubation tests were conducted, and toxicity was observed at the highest doses, demonstrating that the bacteria were exposed to sufficient concentrations of the test material. In the same paper, epoxide metabolites of other chloroalkenes were found to be mutagenic under the same conditions of test (i.e., cis and trans-1-chloropropene oxide, and cis and trans 1,3-dichloropropene oxide). The genotoxicity of these compounds was also assessed by differential growth inhibition of the DNA polymerase-deficient E. coli mutant $polA^-$ compared to its polymerase proficient $polA^+$ parent. TCI oxide was positive in this assay yielding a dose-related decrease in the survival of $polA^-$ cells (up to 40 percent) compared to the $polA^+$ cells, at doses ranging from 0.006 to 0.11 mM/ml.

Both Waskell (1978) and Gu et al. (1981) assessed trichloroethanol for its ability to cause genetic effects. As discussed in the section on gene mutation studies in Salmonella (using TA98 and TA100), Waskell (1978) tested trichloroethanol at doses up to 7.5 mg/plate and found no increases in the revertants, compared to the negative controls.

Gu et al. (1981), on the other hand, found suggestive evidence that trichloroethanol (at 178 mg/l) may be an inducer of SCEs in primary cultures of human lymphocytes. In two experiments (of 24 and 72 karyotypes analyzed, respectively), Gu et al. (1981) found 9.8 ± 3.2 and 9.2 ± 3.3 SCEs/nucleus, compared to the control value of 7.9 ± 3.0.

Trichloroacetic acid and chloral hydrate were also tested by Waskell (1978) in the study mentioned above. Trichloroacetic acid at 0.45 mg/plate was not mutagenic to either TA98 and TA100. Chloral hydrate, however, was weakly active, yielding consistent, but always less than twofold, increases in the number of TA100 revertants, compared to the spontaneous level, over a dose range from 0.5 to 10 mg, both with and without metabolic activation. These increases suggest chloral hydrate is weakly mutagenic to Salmonella strain TA100.

In their testing of chloral hydrate, Gu et al. (1981) found that chloral hydrate at 54.1 mg/l caused increases in the number of SCEs in cultured human lymphocytes, compared to untreated control cells (i.e., 10.7 ± 4.0 versus 7.9 ± 3.0). Again, the response was merely suggestive of an effect.

7.6 BINDING TO DNA

Stott et al. (1982) studied the pharmacokinetics and macromolecular interactions of TCI in B6C3F1 male mice and male Osborne-Mendel rats. As part of their study, mice were given 1200 mg/kg [^{14}C] TCI (>500 uCi/animal, specific activity = 2.4 mCi/mmol) in corn oil. Five hours later, the animals were killed, their livers were removed and frozen, and DNA was extracted two days later. After enzymatic digestion, fractions corresponding to nucleosides or purine and pyrimidine bases were separated and identified by high-pressure liquid chromatography. Aliquots were placed in scintillation fluid and the ^{14}C content was determined by liquid scintillation counting. Analysis of the data indicated that the ability of TCI to alkylate DNA was low. A maximum estimate of the average DNA alkylation level was 0.62 ± 0.42 alkylations/10^6 nucleotides. These data suggest TCI or its metabolites are capable of binding to DNA, but only to a limited extent, and are consistent with the suggestive mutagenic responses reported for TCI and certain of its metabolites.

7.7 SUMMARY AND CONCLUSIONS

Commercially available samples of TCI have been shown to cause responses suggestive of a positive effect in gene mutation studies using bacteria, fungi, higher plants, and somatic cells of mice *in vivo*. These responses occurred with metabolic activation only, suggesting the involvement of one or more metabolites of TCI. This requirement for metabolic activation argues against the possibility that epoxide stabilizers found in many commercial samples are responsible. The marginally increased incidences of revertant counts were only observed at high doses. Thus, commercially available TCI is only weakly mutagenic at most. TCI was not shown to cause structural chromosomal aberrations in the one test conducted to assess this end point.

Other tests provide evidence that commercially available TCI damages DNA. Suggestive and weak-positive responses have been observed in yeast (gene conversion and mitotic recombination), mice (UDS), and humans (SCE and UDS). Metabolic activation was required to obtain the positive responses. Certain metabolites of TCI have been tested for their mutagenic potential, and suggestive positive effects have been shown. TCI or a metabolite(s) may be capable of binding to DNA, but only minimally.

TCI causes weak increases in morphologically abnormal sperm providing evidence that its reaches the gonads. A synopsis of the results of these studies is presented in Table 7-9.

The available data provide suggestive evidence that commercial-grade TCI is a weakly active indirect mutagen, causing effects in a number of different test systems representing a wide evolutionary range of organisms, including humans. Based on this, commercial TCI may have the potential to cause weak or borderline increases above the spontaneous level of mutagenic effects in exposed human tissue. The observation that TCI causes adverse effects in the testes of mice suggests TCI may cause adverse testicular effects in man, too, provided that the pharmacokinetics of TCI in humans also results in its distribution to the gonads. The data on pure TCI do not allow a conclusion to be drawn about its mutagenic potential. However, mutagenic potential cannot be ruled out. If it is mutagenic, the available data suggest TCI would be a very weak, indirect mutagen.

TABLE 7-9. SUMMARY OF TESTS FOR MUTAGENICITY OF TCI

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
I. Gene Mutations	Salmonella typhimurium	Reverse mutations in vitro	Technical grade	-	No control to test effectiveness of S9 mix. No precautions to prevent evaporation.	Henschler et al., 1977
		Plate incorporation tests	Technical grade	+	Twofold increase	Margard, 1978
			Purified	-		Margard, 1978
			Anesthetic grade	-		Waskell, 1978
			Purified[a]	*	1.8-fold increase	Bartsch et al., 1979
		Vapor exposure	Purified[a]	*	1.3-fold increase	Baden et al., 1979
			Reagent[a] grade	*	1.7-fold increase	Simmon et al., 1977
	Escherichia coli	Forward and reverse mutations	Analytical grade	*	Positive for reverse mutations only at arg locus (twofold increase).	Greim et al., 1975
	Schizosaccharomyces pombe	Forward mutations (Host-mediated assays)	Technical grade	*	1.7-fold increase	Loprieno et al., 1979

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
	Saccharomyces cerevisiae	Reverse mutations (*in vitro*)	Purified	-		Loprieno et al., 1979
			Purified	-	Epichlorohydrin and epoxybutane were also negative.	Rossi et al., 1983
			Purified[a]	-		Mondino, 1979
			Technical grade	x	High toxicity	Shahin and Von Borstel, 1977
			ACS reagent grade	+	Fourfold increase both host-mediated assay and liquid suspension test.	Bronzetti et al., 1978
			Technical grade	+	Twofold increase	Callen et al., 1980
	Tradescantia	Forward mutations	Unknown	*		Schairer et al., 1978
	Drosophila melanogaster	Sex-linked recessive lethals	Technical grade	-		Abrahamson and Valencia, 1980
			Technical grade	-		Beliles et al., 1980
	Mouse	Spot test	Technical grade	+	Sixfold increase	Fahrig et al., 1977

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
II. Chromosomal Aberrations	*Drosophila melanogaster*	Chromosome loss	Technical grade	-		Beliles et al., 1980
	Rat	Bone marrow	Technical grade	x	Positive control given by different route of exposure. Doses of TCI may have been too low.	Beliles et al., 1980
	Mouse	Dominant lethal	Purified[a]	-		Slacik-Erben et al., 1980
		Micronucleus	Analytical grade	x	Positive response reported by authors may be due to artifacts in mature erythrocytes.	Duprat and Gradiski, 1980
	Human	Breaks	Occupational exposure	-		Konietzko et al., 1978
		Hypodiploid cells	Occupational exposure	x	Unmatched control group. Hypodiploid cells can be caused by preparation of chromosomes.	Konietzko et al., 1978
III. Other Studies Indicative of Mutagenic Damage	*Saccharomyces cerevisiae*	Gene conversion	ACS reagent grade	+	Twofold increase with metabolic activation.	Bronzetti et al., 1978

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
	Mouse		Technical^a grade	+	Fivefold increase	Callen et al., 1980
		Mitotic recombination	Technical^a grade	+	Fourfold increase	Callen et al., 1980
		Sister chromatid exchange	Anesthetic grade	-	No positive controls. No evidence of toxicity.	White et al., 1979
	Human	Sister chromatid exchange	Occupational exposure	*	Increases correlated with presence of TCI metabolites trichloroethanol and trichloroacetic acid in the blood	Gu et al., 1981
	Rat	Unscheduled DNA synthesis	Technical grade	*	1.5 to 1.8-fold increases	Beliles et al., 1980
			Technical grade	x		Perocco and Prodi, 1981
		HPC DNA repair assay	Stabilized	-	Vinyl chloride only weakly active.	Williams and Shimada, 1983
			Unstabilized	-		Williams and Shimada, 1983
			Technical grade	-		Williams, 1983

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
	Mouse	HPC DNA repair assay	Technical grade	+	8- to 20-fold increases	Williams, 1983
IV. Evidence TCI Reaches the Gonads	Mouse	Morphological sperm abnormalities	Anesthetic grade	*	1.8-fold increase	Land et al., 1979
			Technical grade	+	Threefold increase	Belliles et al., 1980
V. Mutagenicity of Metabolites						
A. TCI-oxide	Salmonella typhimurium	Reverse mutations		-		Kline et al., 1982
	Escherichia coli	Reverse mutations		-		Kline et al., 1982
		Differential killing of repair deficient bacteria		+	40% decreases in survival of Pol- vs. Pol+ cells	Kline et al., 1982
B. Trichloroethanol						
	Salmonella typhimurium	Reverse mutations		-		Waskell, 1978
	Human lymphocytes	Sister chromatid exchange		*		Gu et al., 1981

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
C. Trichloroacetic Acid						
	Salmonella typhimurium	Reverse mutations		-		Waskell, 1978
D. Chloral Hydrate						
	Salmonella typhimurium	Reverse mutations		*	1.6-fold increase	Waskell, 1978
	Human lymphocytes	Sister chromatid exchange		*		Gu et al., 1981

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

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8. CARCINOGENICITY

8.1 DESCRIPTION AND ANALYSIS OF ANIMAL STUDIES AND CELL TRANSFORMATION STUDIES

There have been a number of laboratory investigations of the carcinogenic potential of trichloroethylene (TCI) in experimental animals. These studies have been done using rats, mice, and hamsters, with TCI administered by inhalation, gavage, subcutaneous injection, and topical application. The results are summarized in Table 8-1. Of the studies done, the evidence for the carcinogenicity of TCI consists of statistically significant increases of hepatocellular carcinomas in male and female B6C3F1 mice [National Toxicology Program (NTP), 1982; National Cancer Institute (NCI), 1976; Bell et al., 1978], malignant lymphomas in female NMRI mice (Henschler et al., 1980), and renal adenocarcinomas, significant by life table and incidental tumor tests, in male Fischer 344 rats (NTP, 1982). However, inadequacies in the NTP study in rats and the IBT study in mice and limitations in the interpretation of the data in the Henschler et al. study in mice are evident, as discussed herein.

8.1.1 Oral Administration (Gavage): Rat

8.1.1.1 National Toxicology Program (NTP, 1982)--A carcinogenicity bioassay on TCI in Fischer 344 rats was recently completed for the National Toxicology Program, and a 1982 draft report of the study has been reviewed by its Board of Scientific Counselors.

The TCI test sample was described as a high-purity "Hi-Tri" product obtained in two lots, one being Dow Chemical Company Lot No. TB05-206AA, used for a preliminary dose-finding study and for the first 7 months of the bioassay, and the other being Missouri Solvents Lot No. TB08-039AA, used for the duration of the bioassay. Samples of TCI were chemically analyzed by gas-liquid chromatography,

TABLE 8-1. TCI CARCINOGENICITY BIOASSAYS IN ANIMALS

Study	TCI chemical purity	Species	Dose levels, route	Results
NTP, 1982	Purified	Mice, B6C3F1 Males Females	1000 mg/kg/day gavage, 103 wk	Treatment-related hepatocellular carcinomas in males and females
		Rats, Fischer 344 Males Females	500, 1000 mg/kg/day gavage, 103 wk	Renal adenocarcinomas in treated males
NCI, 1976	Technical grade	Mice, B6C3F1 Males Females	1119, 2339 mg/kg/day 869, 1739 mg/kg/day gavage, 78 wk	Treatment-related hepatocellular carcinomas in males and females
		Rats, Osborne-Mendel Males Females	549, 1097 mg/kg gavage, 88 wk	Negative
Bell et al., (MCA) 1978	Technical grade	Mice, B6C3F1 Males Females	100, 300, 600 ppm inhalation, 24 mo	Increased incidence of hepatocellular carcinomas in males and females with dose
		Rats, Charles River Males Females	100, 300, 600 ppm inhalation, 24 mo	Negative
Maltoni, 1979	Purified	Rats, Sprague-Dawley Males Females	250, 50 mg/kg gavage, 52 wk	Negative
Henschler et al., 1980	Purified	Mice, Han:NMRI Males Females	100, 500 ppm inhalation, 78 wk	Increased incidence of malignant lymphomas in females
		Rats, Han:Wist Males Females	100, 500 ppm inhalation, 78 wk	Negative
		Hamsters, Syrian Males Females	100, 500 ppm inhalation, 78 wk	Negative

(continued on the following page)

TABLE 8-1. (continued)

Study	TCI chemical purity	Species	Dose levels, route	Results
Van Duuren et al., 1979, 1983	Purified	Swiss mice ICR/Ha Female	1 mg, 3x/wk, 581 d topical	Negative
		Female	1 mg, 3x/wk, 14 d 2.5 ug phorbol myristate acetate, topical 452 d	Negative
		Female	0.5 mg sc/wk, 622 d	Negative
		Female Male	0.5 mg, once wk gavage, 622 d	Negative
	Purified TCI epoxide	Female	1 mg TCI epoxide, 3x/wk, 2.5 μg phorbol myristate acetate, topical, 452 d	Negative
		Female	2.5 mg TCI epoxide 3x/wk, topical for 526 d	Negative
		Female	0.5 mg TCI epoxide, once wk, sc, 547 d	Negative
NTP, 1982	Purified	Rats, Osborne-Mendel Marshall 540, August 28807, ACI	Gavage, 104 wk	In preparation
Maltoni, 1979, 1985	Purified	Mice, B6C3F1 Swiss albino	Inhalation, 78 wk	In press
		Rats, Sprague-Dawley	Inhalation, 104 wk	
Henschler et al., 1984	Purified and stabilized	Swiss mice ICR/Ha	Gavage, 78 wk	Negative

mass spectroscopy, infrared spectrophotometry, and nuclear magnetic resonance spectroscopy. Analytical results showed a TCI purity of greater than 99.9%. Epichlorohydrin was not found by gas chromatography-mass spectroscopy at a detection level of 0.001% (v/v). The TCI product contained 8 ppm of diisopropylamine as a stabilizer. Refrigeration at 4°C created no analytically detectable decrease in purity over the course of product usage.

Stock solutions of TCI in corn oil were made once weekly for the first 16 weeks and once monthly for the remainder of the bioassay. Analysis by gas chromatography showed that these solutions were stable for 7 days at room temperature and for 4 weeks at 4°C. Stock solution levels of TCI were within 10% of nominal concentrations.

Based on survival, body weights, and pathology in a preliminary 13-week dose-finding study, estimated maximally tolerated and one-half maximally tolerated doses were selected for the bioassay. For the bioassay, 50 males and 50 females per group were given nothing (untreated controls), corn oil alone (vehicle-controls), 500 mg/kg/day of TCI in corn oil (low dose), or 1,000 mg/kg/day of TCI in corn oil (high dose). Rats were dosed daily, 5 days per week, for 103 weeks. The volume of administered corn oil was 1 mL.

Rats were randomly assigned to dose groups when 8 weeks old at the start of the study. The animals were caged in groups of 5, and decedents were replaced during the first 1.5 weeks. Temperature and humidity in the animal quarters were 22°C to 24°C and 40% to 60%, respectively; room air was exchanged 10 to 15 times every hour.

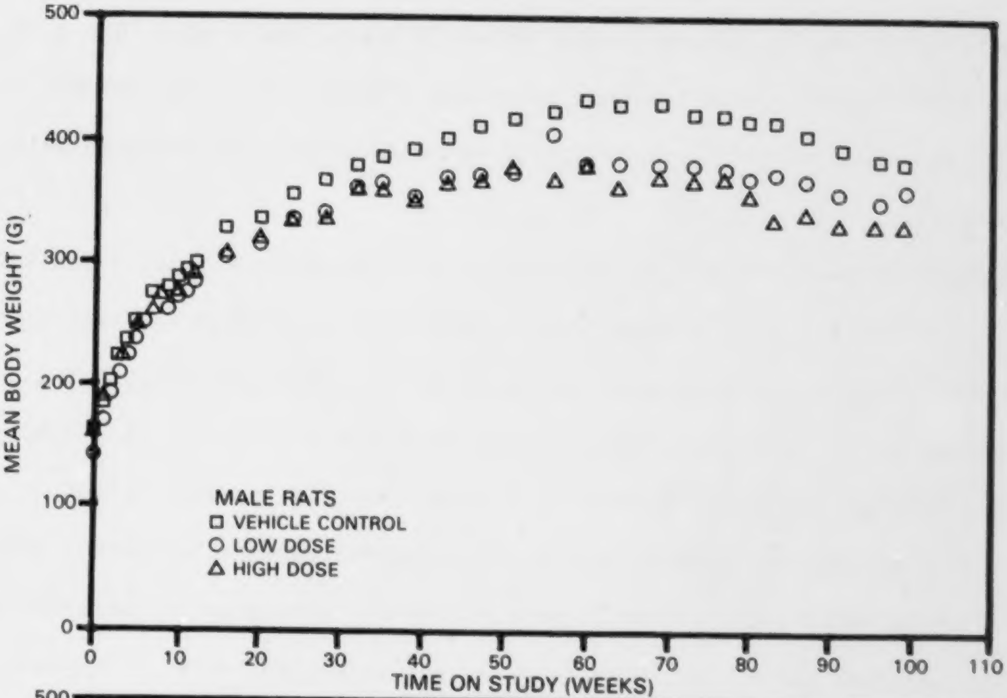
The animals were observed daily. Body weights were recorded weekly for the first 12 weeks and monthly thereafter. Each animal was necropsied, and tissues and organs, as well as all lesions and tumors, were evaluated histopathologically.

Terminal sacrifice of survivors occurred at 111 to 115 weeks.

Body weight trends in Figure 8-1 show reduced mean weight gains in high-dose males and in both treatment groups of females. A dose-related effect on mean body weights in females became evident after 60 weeks, and mean weight gain between vehicle-control and low-dose males was similar (mean body weights at the start of the study were 161 and 141 g for vehicle-control and low-dose males, respectively).

A dose-related reduction in survival of treated male rats and a smaller decrease in survival of high-dose females were evident through survival probabilities estimated by the Kaplan and Meier (1958) technique (Figure 8-2). Differences in survival among female groups were not significant ($p < 0.05$); however, survival was significantly ($p < 0.05$) lower in low-dose males ($p = 0.005$) and high-dose males ($p = 0.001$) compared to vehicle-control males. The following numbers of rats which were killed by "gavage error" were censored from the survival curves in Figure 8-1: 1 vehicle-control male, 3 low-dose males, 10 high-dose males, 2 vehicle-control females, 5 low-dose females, and 5 high-dose females. The numbers of rats alive at terminal sacrifice were as follows: 35/50 vehicle-control males, 20/50 low-dose males, 16/50 high-dose males, 36/50 vehicle-control females, 33/50 low-dose females, and 26/50 high-dose females.

It is indicated in the NTP (1982) report that, because of reduced survival in treated male rats, the statistical methods used which adjust for intercurrent mortality (life table analysis, incidental tumor test) would provide more meaningful results, statistically, than unadjusted statistical methods (Fisher Exact Test, Cochran-Armitage Trend Test). Kidney tumor data in Table 8-2 show a significant ($p < 0.05$) increase in kidney tubular adenocarcinoma incidence in high-dose males (3/16) compared to vehicle-control males (0/33) at terminal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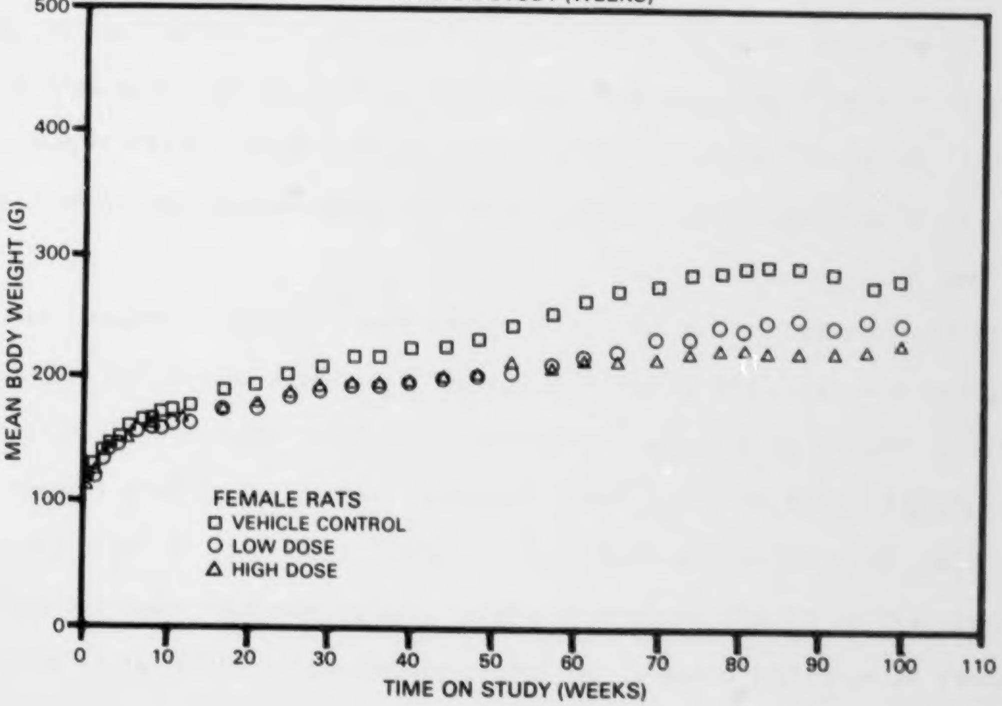
TIME ON STUDY (WEEKS)
MALE RATS
□ VEHICLE CONTROL
○ LOW DOSE
△ HIGH DOSE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△ HIGH DOSE

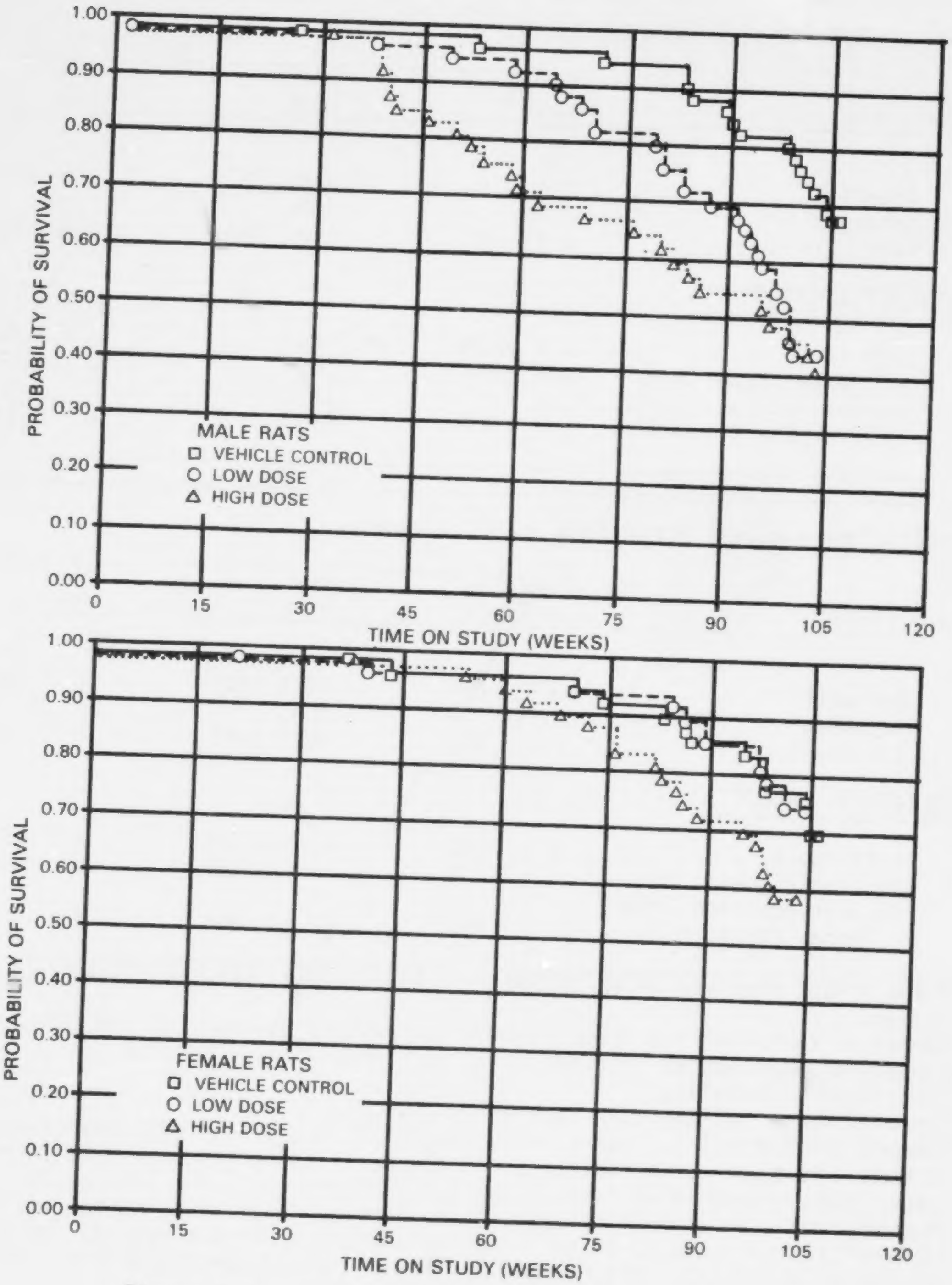

Figure 8-2. Survival curves for rats administered trichloroethylene in corn oil by gavage.

SOURCE: NTP, 1982.

TABLE 8-2. ANALYSIS OF PRIMARY TUMORS IN MALE RATS[a]

KIDNEY: TUBULAR-CELL ADENOCARCINOMA

Tumor rates	Vehicle-control	Low dose	High dose
Overall[b]	0/48 (0%)	0/49 (0%)	3/49 (6%)
Adjusted[c]	0.0%	0.0%	18.8%
Terminal[d]	0/33 (0%)	0/20 (0%)	3/16 (19%)
Statistical tests[e]			
Life table	p = 0.009	(f)	p = 0.028
Incidental tumor test	p = 0.009	(f)	p = 0.028
Cochran-Armitage Trend, Fisher Exact Tests	p = 0.038	(f)	p = 0.125

KIDNEY: TUBULAR-CELL ADENOMA OR ADENOCARCINOMA

Tumor rates	Vehicle-control	Low dose	High dose
Overall[b]	0/48 (0%)	2/49 (4%)	3/49 (6%)
Adjusted[c]	0.0%	5.6%	18.8%
Terminal[d]	0/33 (0%)	0/20 (0%)	3/16 (19%)
Statistical tests[e]			
Life table	p = 0.019	p = 0.194	p = 0.028
Incidental tumor test	p = 0.030	p = 0.327	p = 0.028
Cochran-Armitage Trend, Fisher Exact Tests	p = 0.084	p = 0.253	p = 0.125

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[a] Dosed groups received doses of 500 or 1,000 mg/kg of trichlorethylene by gavage.

[b] Number of tumor-bearing animals/number of animals examined at the site.

[c] Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

[d] Observed tumor incidence at terminal kill.

[e] Beneath the control incidence are the p-values associated with the trend test. Beneath the dosed group incidence are the p-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage Test and the Fisher Exact Test compare the overall incidence rates directly.

[f] The configuration of tumor incidences precludes the use of this statistic.

SOURCE: NTP, 1982.

sacrifice by life table ($p = 0.028$) and incidental tumor ($p = 0.028$) tests. Each test for linear trend was significant ($p < 0.05$), as shown in Table 8-2. Historical corn oil control data for male Fischer 344 rats evaluated in the NTP bioassay program as of June 15, 1981 for studies of at least 104 weeks showed a renal tumor incidence of 3/748 (0.4%) adenocarcinomas; thus, this tumor type is rare in this strain of rats. However, the comparison for kidney adenocarcinomas between high-dose and vehicle-control male rats was not significant ($p < 0.05$) by the Fisher Exact Test, and the statistical significance achieved with the other tests above reflects the significantly ($p \leq 0.005$) higher mortality in high-dose males as compared to vehicle-control males. Other observed kidney tumors in rats include a transitional cell carcinoma of the renal pelvis in a low-dose male, tubular adenomas in two low-dose males, a carcinoma (NOS) of the renal pelvis in a high-dose male, a transitional cell papilloma of the renal pelvis in an untreated control male, and a tubular adenocarcinoma in a high-dose female.

Renal tubular adenocarcinomas had a general histopathologic appearance of solid sheets of cells, often atypical, and small areas of necrosis. These tumors commonly invaded surrounding normal tissue. Small renal tubular adenomas consisted of solid collections of tubular epithelial cells filling contiguous tubules. In larger adenomas there was greater evidence of cellular atypia, and there was no sharp demarcation between larger adenomas and adenocarcinomas. Renal adenocarcinomas were distinguished from renal adenomas by basement membrane invasion.

Toxic nephrosis characterized as cytomegaly was found in 98 treated male rats and 100 treated female rats, but not in control rats. This lesion was found in early deaths, and it progressed in severity during the course of the study. Enlargement of tubular cells was detected early and progressed to the extent that some tubules were dilated and hard to identify. With longer survival the number of dilated cells decreased, corresponding tubules became dilated, and the basement membrane had a striped appearance. The most advanced stage of cytomegaly extended into the renal cortex. Cytomegaly was graded as: 1 - slight, a subtle change in a limited area of the kidney; 4 - severe, an obvious lesion which involved a substantial part of the kidney, and which could alter its function; 2 - moderate; 3 - well marked. Average grades for each group of rats were: 0 for male and female controls, 2.8 for low-dose males, 3.1 for high-dose males, 1.9 for low-dose females, and 2.7 for high-dose females. In contrast, renal nephropathy, a common spontaneous lesion in aging rats, was of lower incidence in treated males (88% in untreated controls, 85% in vehicle controls, 59% in low-dose, and 37% in high-dose) which could relate to the higher mortality in treated males.

Increases of tumor incidences at other sites in treated rats were not apparent. Malignant mesothelioma incidence was higher in low-dose males [vehicle-controls, 1/50; low-dose, 5/50 ($p = 0.042$, life table test); high-dose, 0/48], but high-dose animals did not develop this tumor type, and the low-dose incidence was not significantly ($p < 0.05$) increased according to the other statistical tests used in this study.

The TCI doses used in this study produced statistically significant decreases in the survival of male rats, and a decreased survival in high-dose female rats compared to matched vehicle-controls was apparent, although this difference was not found to be statistically significant. By definition (NCI, 1976) the maximum tolerated dose in carcinogenicity bioassays is the highest dose that can be administered during the chronic study which will not be expected to alter the animals' survival rate from effects other than carcinogenicity. Thus, TCI doses beyond that maximally tolerated, particularly in male rats, appear to have been used in this study. The toxicity of TCI in the kidney tubule region, as cytomegaly, approximated the "well-marked" grade on the average in both groups of treated male rats and the high-dose group of female rats, and it has been noted that the severity of this lesion increased with longer survival. Although a small but statistically significant excess of renal tubular adenocarcinomas in high-dose males compared to vehicle-control males was calculated by statistical methods which adjusted for mortality, the NTP and its Board of Scientific Counselors have concluded that this study in Fischer 344 rats is inadequate for a judgment on the carcinogenicity of TCI. The following is quoted from the latest version (received May 24, 1983) of the abstract on this study from the NTP:

> Under the conditions of these studies, epichlorohydrin-free trichloroethylene caused renal tubular-cell tumors in male F344/N rats, produced nephropathy in both sexes, shortened the survival times of males and did not produce a carcinogenic response among females. This experiment was considered to be inadequate to evaluate the presence or absence of a carcinogenic response to trichloroethylene in male F344/N rats.

Use of additional lower doses in this study could have provided a broader evaluation of dose-response for the observed toxic effects of TCI.

8.1.1.2 National Cancer Institute (NCI, 1976)--A carcinogenicity study of TCI in Osborne-Mendel rats was reported by the National Cancer Institute (NCI) in 1976. The composition of the TCI sample used, determined by vapor-phase chromatography-mass spectrometry, was as follows: TCI, $\geq$ 99.0% (w/w); 1,2-epoxybutane, 0.19%; ethyl acetate, 0.04%; N-methylpyrrole, 0.02%; diisobutylene, 0.03%; epichlorohydrin, 0.09%. The rats were 48 days old when the study began. Treatment groups included 50 animals/sex/dose, and 20 animals/sex comprised matched vehicle-control (corn oil) groups. Ninety-nine male and 98 female rats used as vehicle "colony controls" were also compared with treatment groups in this study. Vehicle "colony controls" included matched controls for TCI and other chemicals being tested concurrently.

Maximum and one-half maximum tolerated doses for the carcinogenicity study were estimated from survival, body weight, and necropsy results from a preliminary 8-week subchronic test. In the carcinogenicity study, treated male and female rats were given time-weighted average doses, by gavage, of 549 and 1,097 mg/kg/treatment of TCI 5 days/week for 78 weeks. Doses were presented as time-weighted averages due to the dose level changes made during the study, as shown in Table 8-3, on the basis of apparent toxicity from treatment with TCI.

Rats were allowed to survive until terminal sacrifice at 32 weeks post-treatment. Animals were observed daily for mortality and toxic signs, and body weights and food consumption were recorded weekly for the first 10 weeks and monthly thereafter. Survivors and decedents were necropsied, and tissues and organs, including tumors and lesions, were examined histopathologically.

At the beginning of the study, rats were randomly assigned to treatment and control groups according to body weight. Body weight trends in Tables 8-4 and 8-5 show decreased body weight gain in males and females in both treatment groups compared to matched vehicle-controls. Food consumption trends in Tables 8-4 and 8-5 indicate decreased food intake by both groups of treated female rats as compared to matched vehicle-controls, whereas food intake by treated male rats appeared comparable to that of matched vehicle-controls. Although decreased body weights in treated animals could reflect decreased food consumption, it would appear that decreases in food consumption and body weights ultimately were a result of toxicity from treatment with TCI. For example, food consumption between matched control and low-dose males was approximately equal, whereas body weight gain was less in the latter group.

TABLE 8-3. DOSAGE AND OBSERVATION SCHEDULE - TRICHLOROETHYLENE CHRONIC STUDY ON OSBORNE-MENDEL RATS

Dosage group	Dose (mg TCI/kg body wt)	Percent of TCI in corn oil	Age at dosing[a] (weeks)	Treatment period (weeks)	Time-weighted av. dose[b] (mg TCI/kg body wt)
Low-dose males and females	650	60.0	7	7[c]	
	750	60.0	14	9[c]	
	500	60.0	23	14[c]	
	500	60.0	37	48[d]	
	no treatment		85	32	549
High-dose males and females	1300	60.0	7	7[c]	
	1500	60.0	14	9[c]	
	1000	60.0	23	14[c]	
	1000	60.0	37	48[d]	
	no treatment	60.0	85	32	1097

Matched controls received doses of corn oil by gavage calculated on the basis of the factor for the high dose animals.

[a] Age at initial dose or dose change.

[b] Time-weighted average dose = $\sum$ (dose in mg/kg x no. of days at that dose)/ $\sum$ (no. of days receiving any dose). In calculating the time-weighted average dose, only the days an animal received a dose are considered.

[c] Dosing 5 days per week each week.

[d] Dosing 5 days per week, cycle of 1 week of no treatment followed by 4 weeks of treatment. (Animals were treated for 38 weeks of the 48-week period.)

SOURCE: NTP, 1982.

Survival patterns in Figures 8-3 and 8-4 indicate a rather poor survival of animals in this study, particularly in treated animals where a decline in survival was evident early in the study. A dose-related decrease in survival of treated male rats was observed; however, survival of low-dose females was less than that of high-dose females early in the study. Statistical analysis of survival patterns in the NCI (1976) report showed a significant ($p < 0.05$) decrease in the survival of high-dose males ($p = 0.001$) vs. matched control

TABLE 8-4. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - MALE RATS

Time interval (weeks)	Vehicle-control				Low dose				High dose			
	Body weight[a] Mean (g)	Body weight[a] Std. dev. (g)	Food[b] (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed
0	193	15.0	0	20	193	15.8	0	50	194	16.7	0	50
14	504	39.4	153	20	474	40.4	150	50	446	45.3	140	45
26	570	43.3	156	20	533	47.1	158	47	500	47.2	150	43
54	616	50.2	149	20	573	47.6	147	40	513	48.3	134	30
78	559	76.7	153	16	523	67.3	158	31	462	47.2	148	12
110	382	26.9	91	2	383	101.5	114	8	423	45.3	255	3

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

SOURCE: NCI, 1976.

TABLE 8-5. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - FEMALE RATS

	Vehicle-control				Low dose				High dose			
Time interval (weeks)	Body weight[a] Mean (g)	Body weight[a] Std. dev. (g)	Food[b] (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed
0	146	11.4	0	20	144	11.0	0	50	144	9.5	0	50
14	302	33.8	107	20	271	24.4	98	44	262	26.3	104	47
26	351	37.6	124	19	293	28.8	110	34	286	30.8	106	45
54	388	51.3	128	17	315	29.8	120	28	307	38.6	113	37
78	373	58.2	146	16	317	39.9	145	20	317	43.5	135	23
110	326	80.1	119	8	311	86.0	114	13	311	67.7	136	13

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

SOURCE: NCI, 1976.

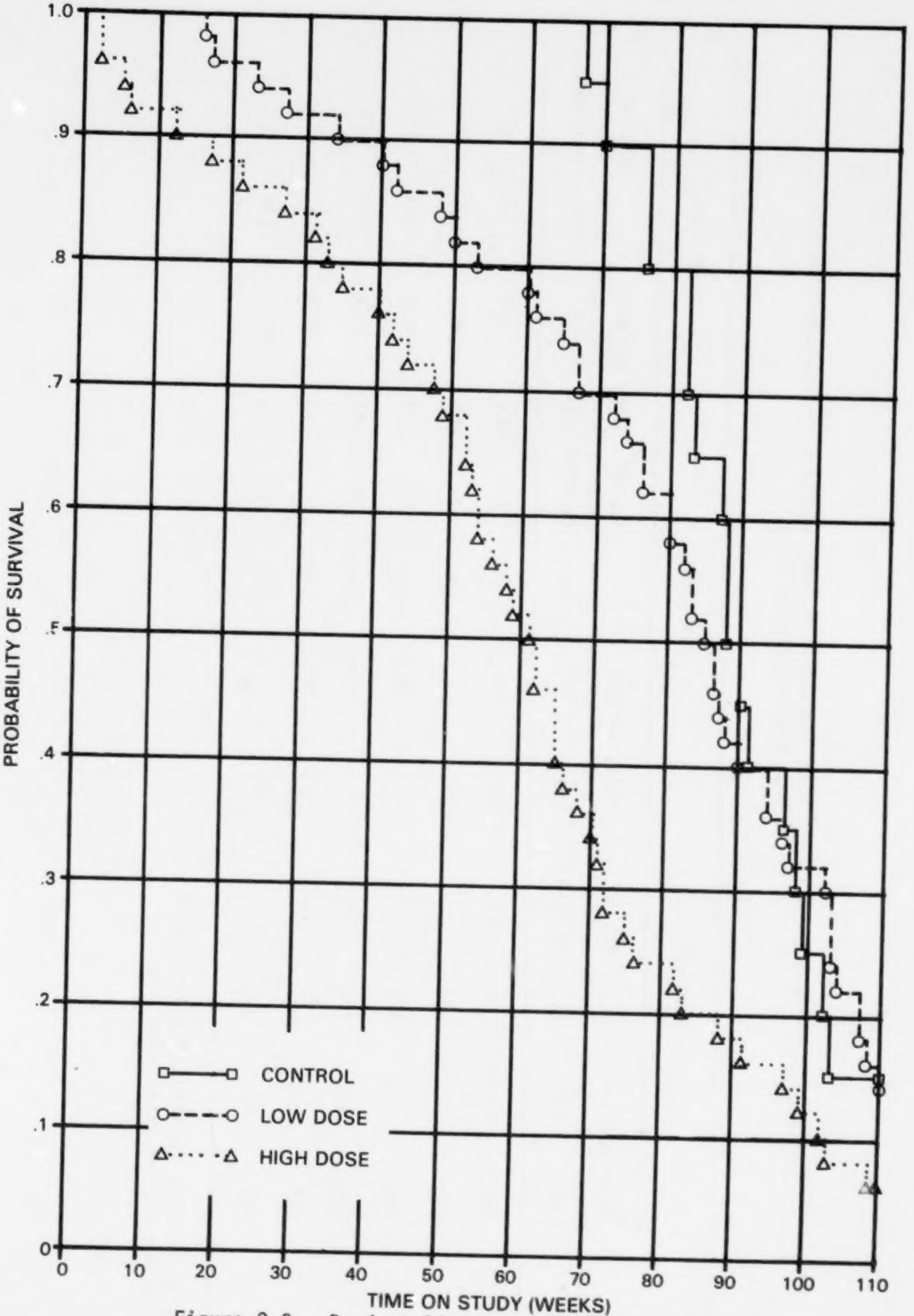

Figure 8-3. Product-limit estimates of probability of survival - trichloroethylene-treated male rats.

SOURCE: NCI, 1976.

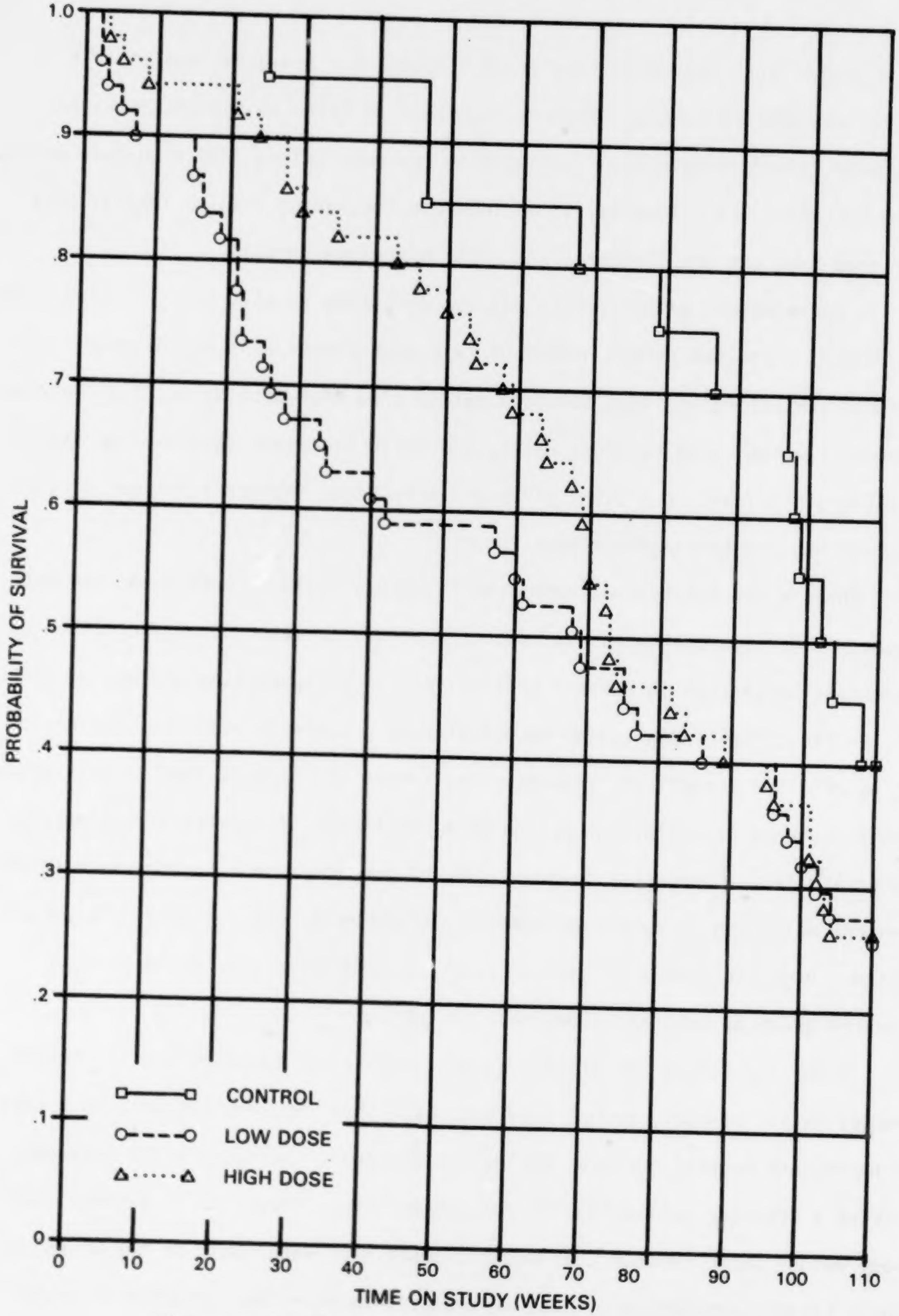

Figure 8-4. Product-limit estimates of probability of survival - trichloroethylene-treated female rats.

SOURCE: NCI, 1976.

males and of both high-dose females ($p = 0.028$) and low-dose females ($p = 0.049$) vs. matched control females. Survival at terminal sacrifice was as follows: 3/20 matched control males, 8/50 low-dose males, 3/50 high-dose males, 8/20 matched control females, 15/48 low-dose females (2 missing females were excluded from the denominator), and 13/50 high-dose females.

A carcinogenic effect of TCI was not discerned in this study. Total tumor incidences expressed as the number of rats with tumors were as follows: 5/20 matched control males, 7/50 low-dose males, 5/50 high-dose males, 7/20 matched control females, 2/48 low-dose males, and 12/50 high-dose females. No statistically significant ($p < 0.05$) differences in tumor incidence between matched control and treatment groups were found.

Chronic nephropathy was common in treated animals of both sexes and both doses. This nephropathy was described as slight to moderate degenerative and regenerative changes in tubular epithelium. This nephropathy was stated to be unlike the chronic nephropathy encountered as a commonly occurring lesion in aging rats and recognized frequently in treated and control rats in this study. The occurrence of unilateral malignant mixed tumors in kidneys of two matched control males, a hamartoma in the kidney of one low-dose male and one matched control male, and an adenocarcinoma in the kidney of one low-dose male were noted. High incidences of chronic respiratory disease were found in each treated group as well as in matched controls.

Under the conditions of this study, TCI was not carcinogenic in Osborne-Mendel rats. However, animal survival was rather low, particularly in treated animals, and greater survival during the course of the study might have permitted a stronger evaluation for carcinogenicity. Reduction in survival and body weight gain indicate that the TCI doses used were toxic to the animals, and a broader evaluation of dose response could have been possible if additional lower doses had been tested and if dose levels had not been adjusted during the study. Rats treated with TCI, and their controls, were housed in a room with other rats treated with dibromochloropropane, ethylene dichloride, 1,1-dichloroethane, or carbon disulfide. There were 10 to 15 air exchanges each hour in the room, but ambient air was not tested for the presence of volatile materials.

8.1.1.3 Maltoni (1979)--Maltoni (1979) summarized the results of a study done to estimate the carcinogenic potential of TCI in Sprague-Dawley rats. Thirty males and 30 females, 13 weeks old, were assigned to each group. Two treatment groups received 50 or 250 mg/kg of TCI by gavage daily, 4 to 5 days weekly for 52 weeks, and a vehicle-control group was given olive oil. The TCI sample was at least 99.9% pure and was contaminated with $\leq$ 50 ppm each of 1,2-dichloroethylene, chloroform, carbon tetrachloride, and 1,1,2-trichloroethane. No detectable epoxides were found in this test material, and 10 ppm diisopropylamine was added as a stabilizer. Surviving animals were sacrificed at 140 weeks. All animals were given a necropsy examination, and tissues and organs were evaluated microscopically.

A carcinogenic effect was not demonstrated in this study. However, although the experiment lasted 140 weeks, the animals were dosed for 52 weeks, which is a duration below potential lifetime exposures. Tumor findings were presented in the study report; however, mortality patterns and toxic signs, if any, were not described to indicate the sensitivity of the animals to the TCI treatment used. Use of animals younger than 13 weeks of age at the beginning of the study could have given a broader assessment of the carcinogenic potential of TCI through an evaluation of treatment with TCI during the early growth and development of the rats.

8.1.2 Oral Administration (Gavage): Mouse

8.1.2.1 National Toxicology Program (NTP, 1982)--A carcinogenicity bioassay on TCI in B6C3F1 mice was recently completed for the National Toxicology Program, and a 1982 draft report of the study has been reviewed by the NTP's Board of Scientific Counselors.

The TCI test sample and the experimental design were those used in the NTP (1982) carcinogenicity bioassay in Fischer 344 rats previously described in this document. Evaluation of mortality, body weights, and pathology in a 13-week preliminary dose-finding study led to the selection of 1,000 mg/kg/day, 5 days per week, for 103 weeks as the single dose level used in the bioassay. Fifty males and 50 females per group were assigned to untreated control groups, corn oil vehicle-control groups, and treatment groups, when 8 weeks old. Vehicle-control and treated animals received 0.5 mL corn oil with each gavage administration. Mice were caged in groups of 10 for the first 8 months of the study, and in groups of 5 for the rest of the study. The animals were evaluated for mortality, body weight, and pathology until terminal sacrifice at 112 to 115 weeks.

Results of this study were analyzed by the statistical methods used for the NTP (1982) carcinogenicity bioassay in rats. Mean body weights of treated males were lower than those of vehicle control males, whereas mean body weights between vehicle control and treated females were comparable (Figure 8-5). Survival was significantly ($p = 0.004$) lower in treated males compared to vehicle-controls, and although survival in treated females was lower after 95 weeks, the overall difference in survival between vehicle-control and treated females was not significant ($p < 0.05$) (Figure 8-6). Deaths of two vehicle-control males, three treated males, one vehicle-control female, and three treated females were attributed to "gavage error," and these animals were censored from the survival

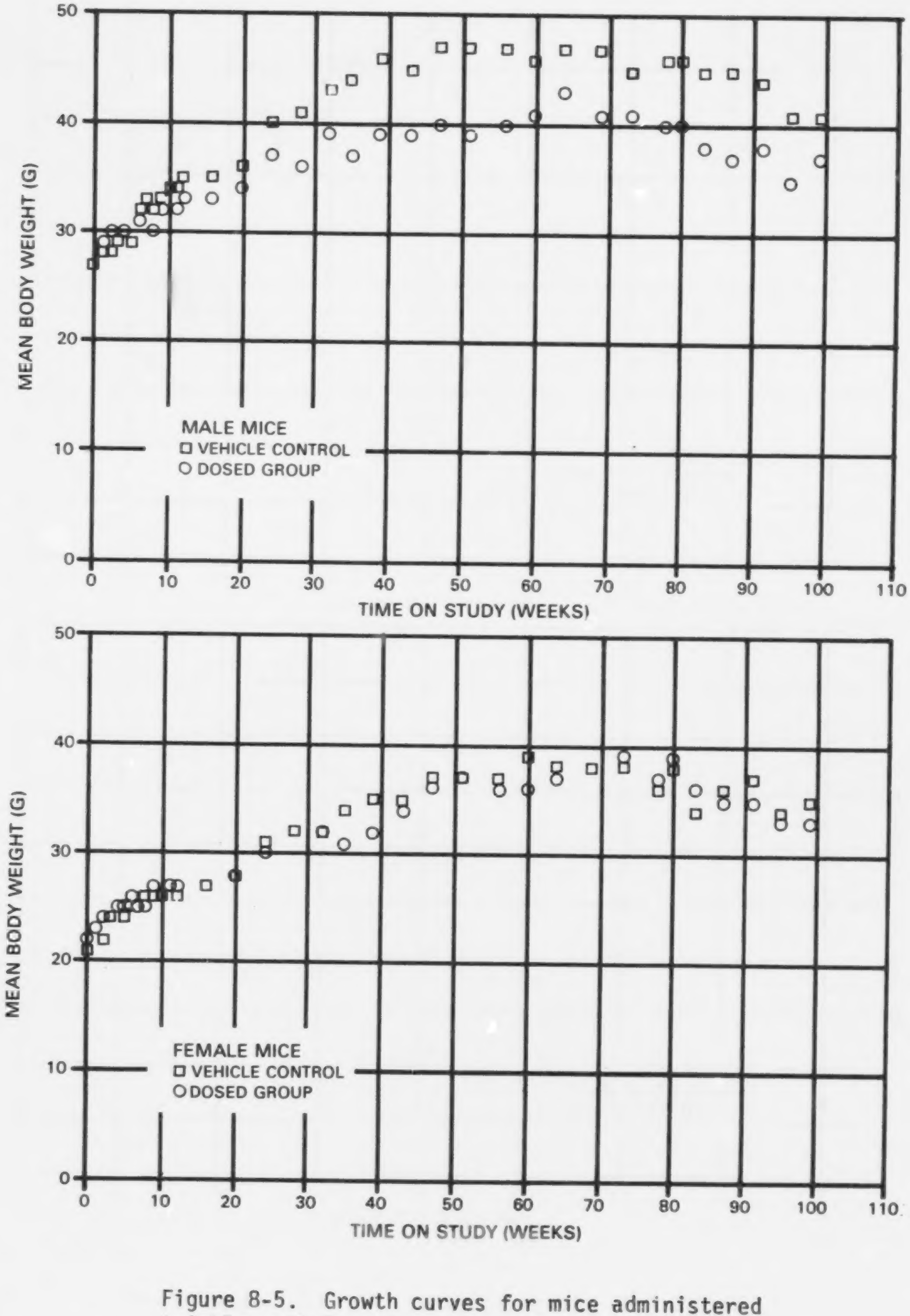

Figure 8-5. Growth curves for mice administered trichloroethylene in corn oil by gavage.

SOURCE: NTP, 1982.

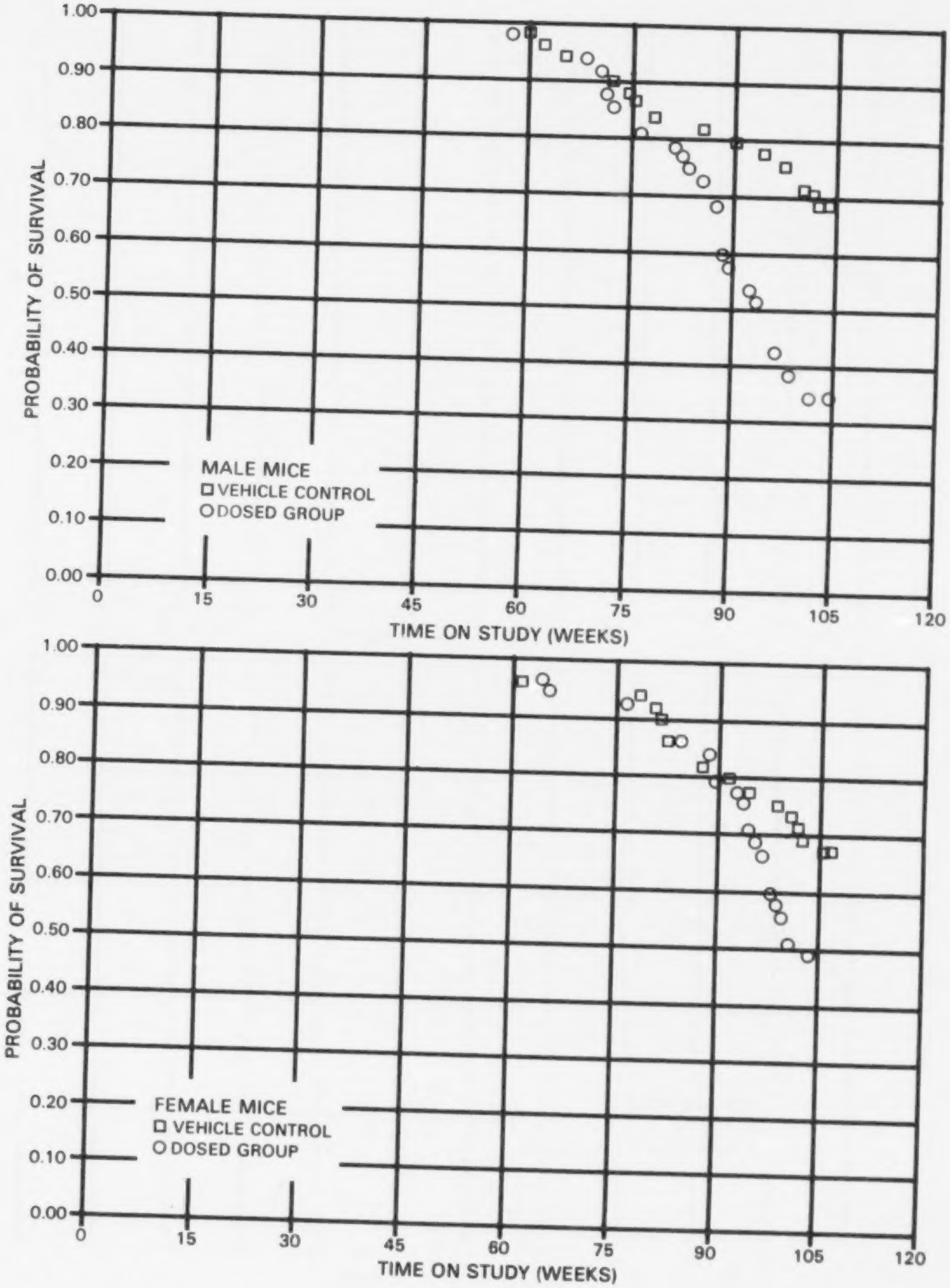

Figure 8-6. Survival curves for mice administered trichloroethylene in corn oil by gavage.

SOURCE: NTP, 1982.

patterns shown in Figure 8-6. At terminal sacrifice, 33/50 vehicle-control males, 16/50 treated males, 32/50 vehicle-control females, and 23/50 treated females were alive.

A carcinogenic effect of TCI was not apparent in this study except for a significant ($p \leq 0.002$) increase in the incidence of hepatocellular carcinomas in treated male and female mice compared to corresponding vehicle-controls (Tables 8-6 and 8-7). Hepatocellular carcinomas metastasized to the lung in five treated males and one vehicle-control male. Hepatocellular adenoma incidence was significantly ($p < 0.05$) increased in treated female mice by each statistical method indicated in Table 8-7, but in treated male mice only by life table analysis (Table 8-6). Spontaneous hepatocellular tumor formation is a characteristic of the B6C3F1 mouse strain, and incidences of hepatocellular carcinoma in historical corn oil control B6C3F1 mice as of June 15, 1981 for carcinogenicity studies of 104 weeks duration in the NTP bioassay program were 18% (120/256) in males and 2.9% (22/751) in females.

Hepatocellular carcinomas had a general histopathologic appearance of markedly abnormal cytology and architecture, with diffuse patterns of tumor tissue infiltrating normal tissue, whereas hepatocellular adenomas consisted of circumscribed areas of distinct parenchyma cells. A treatment-related effect on nonneoplastic pathology in liver was not evident in the study report.

Cytomegaly in the kidney was found in 90% of treated male and 98% of treated female mice, but not in corresponding vehicle-controls. The average grades of severity for cytomegaly, according to the scheme used for grading cytomegaly in kidneys of treated Fischer 344 rats in the previously described NTP (1982) study, were 1.5 in dosed males and 1.8 in dosed females. Comparison with the average grades for male and female Fischer 344 rats given 1,000 mg/kg/day of TCI in the NTP (1982) bioassay indicates that toxic nephrosis of the kidney

TABLE 8-6. ANALYSIS OF PRIMARY TUMORS IN MALE MICE[a]

LIVER: ADENOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	3/48 (6%)	8/50 (16%)
Adjusted[c]	9.1%	27.5%
Terminal[d]	3/33 (9%)	1/16 (6%)
Statistical tests[e]		
Life table		p = 0.022
Incidental tumor test		p = 0.191
Cochran-Armitage Trend, Fisher Exact Tests		p = 0.113

LIVER: CARCINOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	8/48 (17%)	30/50 (60%)
Adjusted[c]	22.1%	92.5%
Terminal[d]	6/33 (18%)	14/16 (87.5%)
Statistical tests[e]		
Life table		p < 0.001
Incidental tumor test		p < 0.001
Cochran-Armitage Trend, Fisher Exact Tests		p < 0.001

LIVER: ADENOMA OR CARCINOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	11/48 (23%)	38/50 (76%)
Adjusted[c]	30.7%	97.1%
Terminal[d]	9/33 (27%)	15/16 (94%)
Statistical tests[e]		
Life table		p < 0.001
Incidental tumor test		p < 0.001
Cochran-Armitage Trend, Fisher Exact Tests		p < 0.001

[a] The dosed group received doses of 1,000 mg/kg of trichlorethylene by gavage.
[b] Number of tumor-bearing animals/number of animals examined at the site.
[c] Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
[d] Observed tumor incidence at terminal kill.
[e] Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Fisher Exact Test compares the overall incidence rates directly.

SOURCE: NTP, 1982.

TABLE 8-7. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE[a]

LIVER: ADENOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	2/48 (4%)	8/49 (16%)
Adjusted[c]	6.2%	30.2%
Terminal[d]	2/32 (6%)	6/23 (26%)
Statistical tests[e]		
Life table		$p = 0.016$
Incidental tumor test		$p = 0.023$
Cochran-Armitage Trend, Fisher Exact Tests		$p = 0.049$

LIVER: CARCINOMA

Tumor rates	Vehicle-control	Dose
Overall[b]	2/48 (4%)	13/49 (27%)
Adjusted[c]	6.2%	43.9%
Terminal[d]	2/32 (6%)	8/23 (35%)
Statistical tests[e]		
Life table		$p < 0.001$
Incidental tumor test		$p = 0.002$
Cochran-Armitage Trend, Fisher Exact Tests		$p = 0.002$

LIVER: ADENOMA OR CARCINOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	4/48 (8%)	19/49 (39%)
Adjusted[c]	12.5%	63.8%
Terminal[d]	4/32 (13%)	13/23 (57%)
Statistical tests[e]		
Life table		$p < 0.001$
Incidental tumor test		$p < 0.001$
Cochran-Armitage Trend, Fisher Exact Tests		$p < 0.001$

[a] The dosed group received doses of 1,000 mg/kg of trichlorethylene by gavage.

[b] Number of tumor-bearing animals/number of animals examined at the site.

[c] Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

[d] Observed tumor incidence at terminal kill.

[e] Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Fisher Exact Test compares the overall incidence rates directly.

SOURCE: NTP, 1982.

was less severe in the mice in this study at an equivalent dose. Only one dosed mouse had a cytomegaly grade above 2. One vehicle-control male and one treated male each had a renal tubular cell adenocarcinoma.

Under the conditions of this bioassay, TCI treatment produced statistically significant increases of hepatocellular carcinomas in male and female B6C3F1 mice. These results repeated those of the NCI (1976) carcinogenicity study discussed herein, showing statistically significant increases in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice given TCI stabilized with epoxides. In effect, the repeat study excludes the epoxides as necessary factors in the increased incidence of hepatocellular carcinomas in male and female B6C3F1 mice treated with TCI under the conditions of the NTP (1982) and NCI (1976) bioassays. Use of additional doses in the NTP (1982) study could have provided an evaluation of dose response for hepatocellular carcinomas in mice.

8.1.2.2 National Cancer Institute (NCI, 1976)--A carcinogenicity study of TCI in B6C3F1 mice was reported by the National Cancer Institute (NCI) in 1976. The TCI sample was that used in the NCI (1976) study in Osborne-Mendel rats described herein. Treatment groups included 50 animals/sex/dose, and 20 animals/sex comprised matched vehicle-control (corn oil) groups. Seventy-seven male and 80 female mice used as vehicle "colony controls" and 70 male and 76 female mice serving as untreated "colony controls" were also compared with the treatment groups in this study; "colony controls" included matched controls for TCI and other chemicals being tested concurrently. Mice were 35 days old when the study began.

Maximum and one-half maximum tolerated doses for the carcinogenicity study were estimated from survival, body weight, and necropsy results from a preliminary

8-week subchronic test. In the carcinogenicity study, time-weighted average doses of TCI were given by gavage, 5 days/week, for 78 weeks, to the mice at levels of 1,169 and 2,339 mg/kg/treatment in males and 869 and 1,739 mg/kg/treatment in females. Doses were presented as time-weighted averages due to the dose level changes made during the study, as shown in Table 8-8, in an attempt to maintain maximum and one-half maximum tolerated doses.

TABLE 8-8. DOSAGE AND OBSERVATION SCHEDULE - TRICHLOROETHYLENE CHRONIC STUDY ON B6C3F1 MICE

Dosage group	Dose (mg TCI/kg body wt)	Percent of TCI in corn oil	Age at dosing[a] (weeks)	Treatment period (weeks)	Time-weighted av. dose[b] (mg TCI/kg body wt)
Low-dose males	1000	15.0	5	6[c]	
	1000	10.0	11	6[c]	
	1200	24.0	17	66[c]	
	no treatment		83	12	1169
High-dose males	2000	15.0	5	6[c]	
	2000	20.0	11	6[c]	
	2400	24.0	17	66[c]	
	no treatment		83	12	2339
Low-dose females	700	10.0	5	12[c]	
	900	18.0	17	66[c]	
	no treatment		83	12	869
High-dose females	1400	10.0	5	6[c]	
	1400	20.0	11	6[c]	
	1800	18.0	17	66[c]	
	no treatment		83	12	1739

Matched controls received doses of corn oil by gavage calculated on the basis of the factor for the high dose animals.

[a] Age at initial dose or dose change.

[b] Time-weighted average dose = $\sum$ (dose in mg/kg x no. of days at that dose)/ $\sum$ (no. of days receiving any dose). In calculating the time-weighted average dose, only the days an animal received a dose are considered.

[c] Dosing 5 days per week each week.

SOURCE: NCI, 1976.

Mice were permitted to survive until terminal sacrifice at 12 weeks following treatment. Animals were observed daily for mortality and toxic signs, and body weights and food consumption were recorded weekly for the first 10 weeks and monthly thereafter. Survivors and decedents were necropsied, and tissues and organs, including tumors and lesions, were examined histopathologically.

At the beginning of the study, mice were randomly assigned to treatment and control groups according to body weight. Body weight trends in Tables 8-9 and 8-10 show comparable weight gain between matched vehicle-controls and both treatment groups for both males and females. Food consumption trends in Tables 8-9 and 8-10 indicate similar consumption between the female groups and slightly higher consumption in treated males as compared to matched controls. Therefore, it is possible that, if food consumption between matched control and treated groups of males had been equivalent, body weight gain in treated males might have been less than that in matched control males.

Survival patterns are presented in Figures 8-7 and 8-8. Differences in survival reported as statistically significant ($p < 0.05$) were those between high-dose and low-dose males ($p = 0.001$), matched control males and low-dose males ($p = 0.004$), and both treatment groups of females and matched control females ($p = 0.035$). Survival at terminal sacrifice was as follows: 8/20 matched control males, 36/50 low-dose males, 22/50 high-dose males, 20/20 matched control females, 42/50 low-dose females, and 42/47 high-dose females (3 high-dose females were missing and not included in the denominator).

Prior to terminal sacrifice, approximately 50% of the treated males and "a few" treated females exhibited bloating or abdominal distension. Results of necropsy and histopathologic examinations revealed a statistically significant ($p < 0.05$) increase in the incidence of hepatocellular carcinomas in male and

TABLE 8-9. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - MALE MICE

Time interval (weeks)	Vehicle-controls				Low dose				High dose			
	Body weight[a]				Body weight				Body weight			
	Mean (g)	Std. dev. (g)	Food[b] (g)	No. of animals weighed	Mean (g)	Std. dev. (g)	Food (g)	No. of animals weighed	Mean (g)	Std. dev. (g)	Food (g)	No. of animals weighed
0	17	0.5	0	20	17	2.0	0	50	17	1.1	0	50
14	28	0.1	23	20	28	0.8	27	50	29	0.4	26	49
26	31	0.2	24	20	31	0.9	28	48	32	0.7	27	42
54	32	0.4	21	18	33	1.0	26	45	34	0.4	30	33
78	34	0.6	24	8	34	0.8	32	40	35	1.0	39	24
90	34	0.7	34	8	33	0.7	32	35	34	1.3	38	20

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

SOURCE: NCI, 1976.

TABLE 8-10. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - FEMALE MICE

Time interval (weeks)	Vehicle-controls				Low dose				High dose			
	Body weight[a]		Food[b] (g)	No. of animals weighed	Body weight		Food (g)	No. of animals weighed	Body weight		Food (g)	No. of animals weighed
	Mean (g)	Std. dev. (g)			Mean (g)	Std. dev. (g)			Mean (g)	Std. dev. (g)		
0	14	0.0	0	20	14	0.6	0	50	14	0.7	0	50
14	23	0.4	27	20	23	0.3	23	49	24	0.5	24	49
26	25	0.2	22	19	26	0.7	23	49	25	0.5	24	47
54	28	1.1	19	18	27	0.5	21	45	26	0.3	22	41
78	29	0.6	23	18	29	0.7	25	41	28	0.4	26	40
90	28	0.6	28	17	30	0.6	27	40	28	0.7	26	39

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

SOURCE: NCI, 1976.

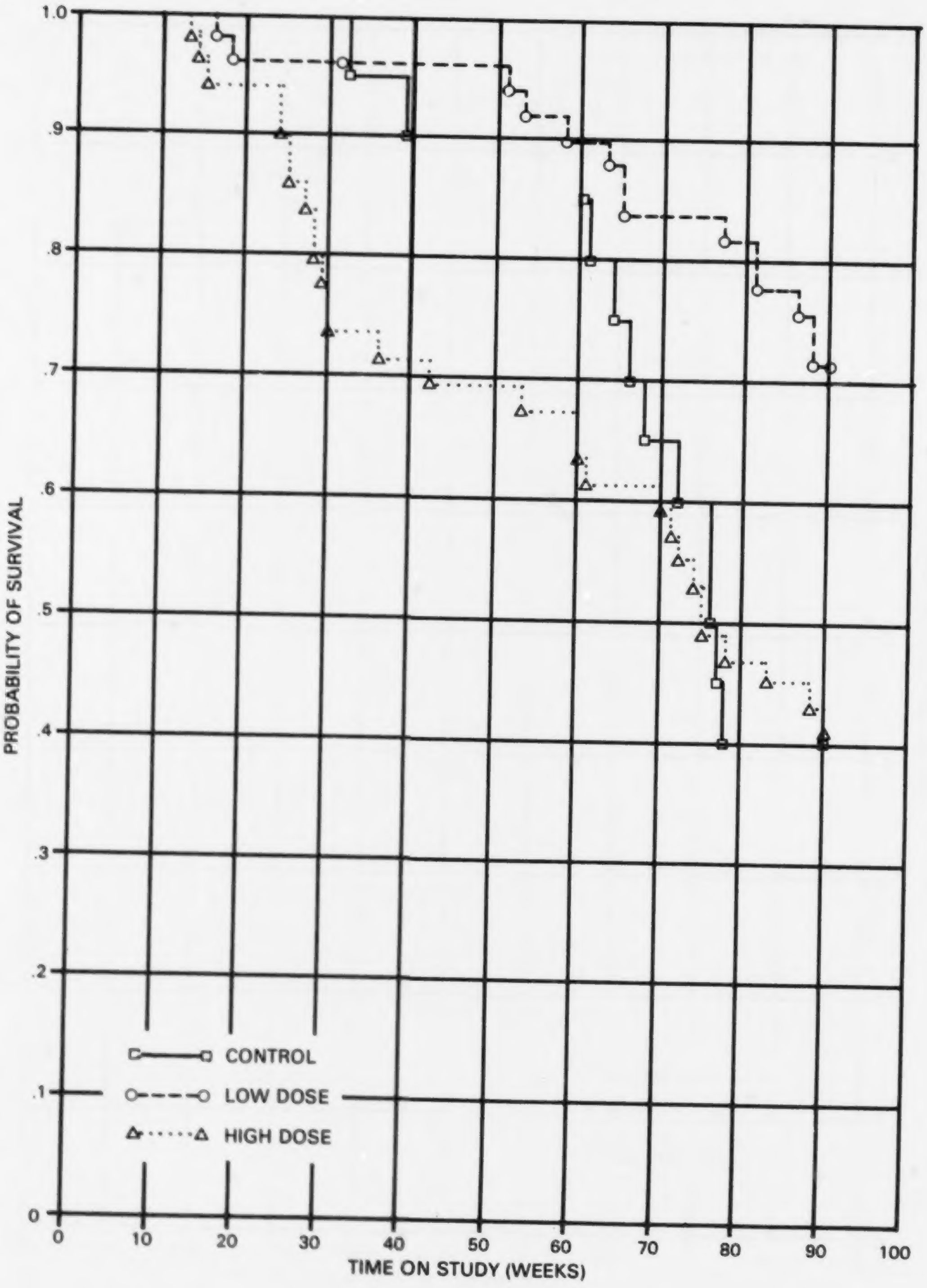

Figure 8-7. Product-limit estimates of probability of survival - trichloroethylene-treated male mice.

SOURCE: NCI, 1976.

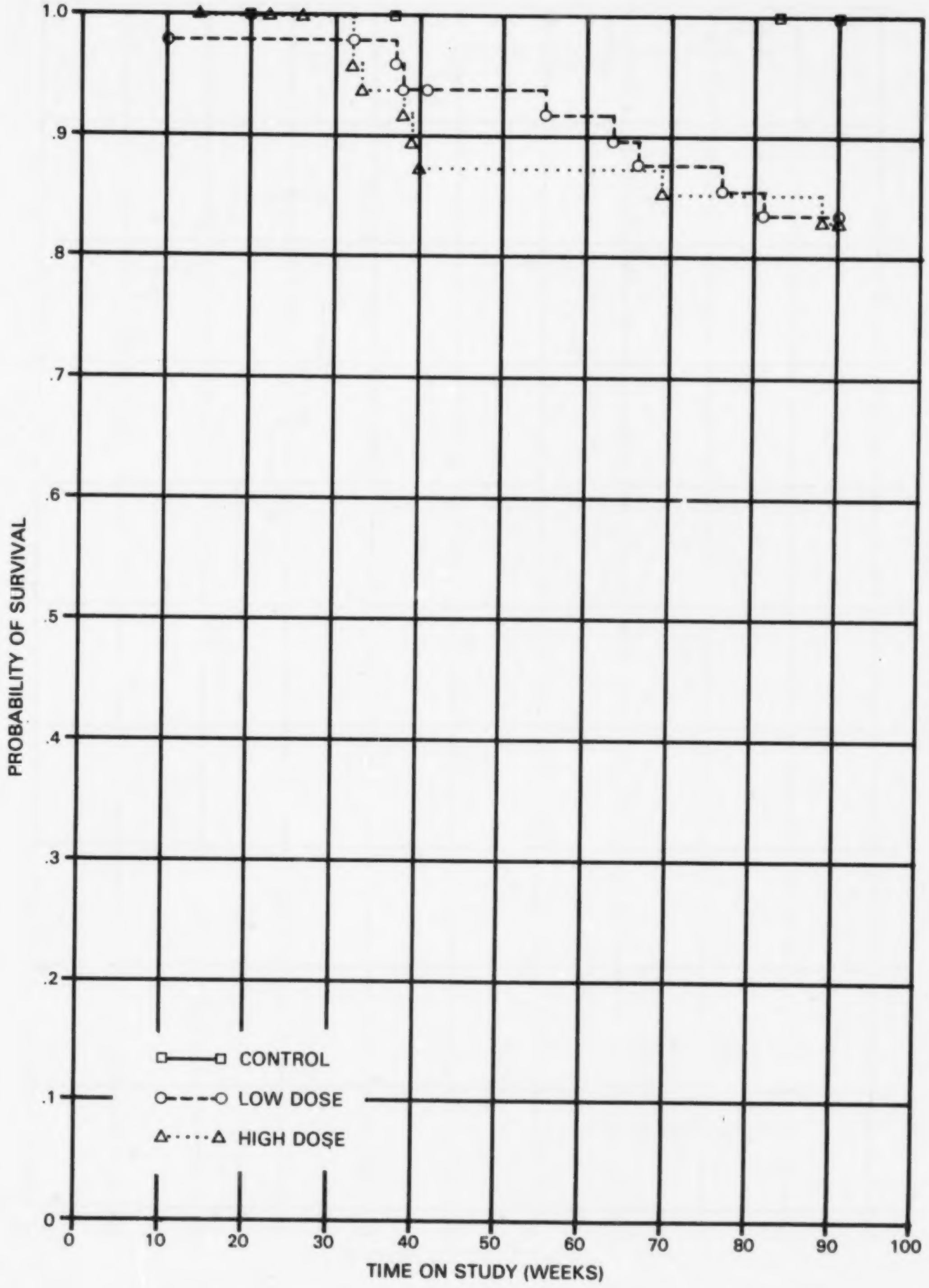

Figure 8-8. Product-limit estimates of probability of survival - trichloroethylene-treated female mice.

SOURCE: NCI, 1976.

female mice treated with TCI (Table 8-11). A dose-related response in females is evident. Metastasis of hepatocellular carcinoma to the lung was observed in four low-dose males and in three high-dose males. The time to observation of the first hepatocellular carcinoma in each group was as follows: 72 weeks in matched control males, 81 weeks in low-dose males, 27 weeks in high-dose males, and 90 weeks in females. With regard to "colony control" mice, hepatocellular carcinomas were diagnosed in 5/77 (6.5%) vehicle-control males, 5/70 (7.1%) untreated control males, 1/80 (1.3%) vehicle-control females, and 2/76 (2.6%) untreated control females. Hepatocellular carcinoma incidences in "colony control" mice were comparable to those in matched vehicle-control males (1/20 or 5%) and females (0/20 or 0%). Statistically significant ($p < 0.05$) differences in the incidence of other tumor types between control and treatment groups were not found.

The general pathology of hepatocellular carcinomas consisted of various characteristics, including an orderly cord-like arrangement of neoplastic cells, a pseudoglandular pattern resembling adenocarcinoma, or sheets of highly anaplastic cells with minimal cord- or gland-like arrangement.

Under the conditions of this study, TCI induced a statistically significant ($p < 0.05$) increase in hepatocellular carcinoma incidence in male and female B6C3F1 mice. Although the number of matched vehicle controls was low, the use of pooled colony controls gives additional support for treatment-related effects.

A more precise estimate of dose response perhaps could have been obtained if additional lower doses had been used and if constant doses rather than time-weighted averages had been used. Treated animals were housed in the

TABLE 8-11. INCIDENCE OF HEPATOCELLULAR CARCINOMAS IN B6C3F1 MICE RECEIVING TRICHLOROETHYLENE BY ORAL GAVAGE

	Males			Females		
Dose	0 mg/kg	1169 mg/kg	2339 mg/kg	0 mg/kg	869 mg/kg	1739 mg/kg
Number with tumors/number of animals	1/20	26/50	31/48	0/20	4/50	11/47
Incidence	0.05	0.52	0.646	0	0.08	0.234
p-value		1.39×10^{-4}[a]	3.44×10^{-6}[a]		1.75×10^{-1}	1.35×10^{-2}[a]
Number with metastases	0/20	4/50	3/48	0/20	0/50	0/47

[a] Statistically significant ($p < 0.05$) increase compared to matched vehicle-controls (0 mg/kg) by Fisher Exact Test.

SOURCE: NCI, 1976.

same room as mice treated with other volatile compounds*; however, since 1) controls were in the same room as treated animals, 2) oral TCI doses probably would have been much higher than ambient levels of other volatiles, 3) the cages had filters to limit the amount of chemical released into the ambient air, 4) the total room air was exchanged 10 to 15 times per hour, and 5) dosing was done in another room under a large hood, the likelihood that the other volatile compounds were responsible for the observed results is considered low. Ambient levels of volatiles in the animal quarters were not measured. Further evidence that the induction of hepatocellular carcinomas in B6C3F1 mice was due to treatment with TCI in this study is the repeat study (NTP, 1982) with a similar experimental design in which the mice were not housed with mice treated with other volatile chemicals. Additionally, the repeat NTP (1982) study of purified TCI in B6C3F1 mice devoid of detectable levels of epoxides indicates that the presence of epoxides was not necessary for the induction of hepatocellular carcinomas in B6C3F1 mice in the NCI (1976) study.

8.1.2.3 Van Duuren et al. (1979)†--A carcinogenicity study of purified TCI in ICR/Ha Swiss mice was described by Van Duuren et al. (1979). Experimental details provided below include those obtained through written communication with B.L. Van Duuren, New York University. The TCI sample was > 99% pure, and, although contaminants were not identified, epoxides were considered not to be present in the test material. It was stated that although range-finding studies were done to estimate maximum tolerated doses (doses which did not affect body weight gain or induce clinical signs of toxicity in a 6- to 8-week test which included histopathologic examination of treatment site sections) for all test groups in this study, TCI doses used in the main topical application experiments were below those which could have been tolerated by the animals. TCI doses used in the skin treatment experiments were selected to permit a comparison with TCI oxide, with respect to carcinogenicity, on an equimolar basis. This study incorporated the following experiments: 1) 30 females received topical applications of 1 mg TCI three times weekly for approximately 581 days; 2) 30 females were treated topically with a single application of 1.0 mg TCI followed 14 days later by applications of 2.5 μg phorbol myristate acetate (PMA) to the skin three times weekly until termination of the study at 452 days. In addition, a different group of 30 females was similarly treated with an application of 1.0 mg TCI epoxide, a metabolite of TCI, followed by applications of 2.5 μg PMA; 3) 30 females were given subcutaneous injections of 0.5 mg TCI once weekly for 622 days; and 4) 30 males and 30 females were administered 0.5 mg TCI by gavage once weekly for 622 days. A vehicle-control group of 30 mice and an untreated control group of 100 mice were included in each experiment. Dosing vehicles were acetone in the skin treatment tests and trioctanoin in the other tests. In the initiation-promotion experiment, 210 mice treated with PMA alone were also on test. The mice were 6 to 8 weeks old at the beginning of the study; six animals were housed in each cage. Test sites on the skin were shaved as necessary and were not covered, and test samples were administered in ventilated hoods; however, it was the authors' impression that TCI was immediately absorbed and that evaporation from test sites was minimal. The animals were weighed monthly, and each animal was examined by necropsy. Tumors and lesions were examined histopathologically. Histopathologic evaluation was routinely done on skin, liver, stomach, and kidney in the skin application experiments, injection site tissue and liver in the subcutaneous injection experiment, and stomach, liver, and kidney in the gavage experiment.

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*1,1,2,2-tetrachloroethane, 3-chloropropene, chloropicrin, 1,1-dichloroethane, chloroform, sulfolene, iodoform, ethylene dichloride, methyl chloroform, 1,1,2-trichloroethane, tetrachloroethylene, hexachloroethane, carbon disulfide, trichlorofluoromethane, carbon tetrachloride, ethylene dibromide, dibromochloropropane.

†Although discussed under the heading of Oral Administration: Gavage (Mouse), this study in mice also includes subcutaneous injection, repeated skin application, and initiation-promotion experiments with TCI and an initiation-promotion experiment with TCI oxide.

Treatment with TCI and TCI oxide did not produce statistically significant ($p < 0.05$) increases in tumor incidence in this study (Tables 8-12, 8-13, and 8-14). In the initiation-promotion experiment, the numbers of mice with skin papillomas (squamous cell carcinomas) were: 4 (1) treated with TCI, 15 (3) treated with PMA alone, and none in the control groups. Repeated application to the skin produced lung and stomach tumors in 9 and 1 TCI-treated mice, respectively, 11 and 2 vehicle-treated controls, respectively, and 30 and 5 untreated controls, respectively. Lung tumors were diagnosed as benign papillomas, and stomach tumors were described as papillomas of the forestomach. Tumors were not found in TCI-treated and control animals in the experiment with subcutaneous doses. In the experiment with gavage dosing, forestomach tumors were observed in one female and two male mice given TCI, no vehicle-control animals, and five female and eight male (one male had a squamous cell carcinoma in the forestomach) untreated control mice. Three mice given repeated applications of TCI oxide onto the skin had skin papillomas. Results in Tables 8-12, 8-13, and 8-14 show that the mice were sensitive to positive control agents under the design of this study. Median survival times approximated the number of days the animals were on test, and body weights were comparable between treatment and control groups in each experiment.

Use of higher TCI doses could have more strongly challenged the mice for carcinogenicity in the skin treatment experiments, but Van Duuren et al. (1983) noted that low activity or lack of activity of chloroolefins tested for carcinogenicity in skin treatment experiments in their laboratory was expected because of evidence for the relatively low level of epoxidizing enzymes in mouse

TABLE 8-12. MOUSE SKIN BIOASSAYS OF TRICHLOROETHYLENE AND TRICHLOROETHYLENE OXIDE (30 FEMALE HA:ICR SWISS MICE PER GROUP)

	Initiation-promotion[a]			Repeated application[b]			
Compound	Dose, mg/ application/ mouse	Days to first tumor	Mice with papillomas/ total papillomas	Dose, mg/ application/ mouse	Days to first tumor	Mice with papillomas/ total papillomas	No. of mice with distant tumors[d]
Trichloroethylene	1.0	264	4/9 (1)[c]	1.0	-	0	9 lung 1 stomach
Trichloroethylene oxide	1.0	371	3/3	NT	-	-	-
7,12-Dimethyl-benz[a]anthracene[e]	0.02	54	29/317 (18) $p < 0.0005$[f]	NT	-	-	-
PMA controls (120 mice) (90 mice)	0.0025 0.005	141 449	9/10 (1) 6/7 (2)				
Acetone (0.1 mL)	-	-	-	0.1 mL	-	0	11 lung 2 stomach
No treatment (100 mice)	-	-	-	-	-	0	30 lung 5 stomach

[a] Compounds were applied to dorsal skin in 0.1 mL acetone once followed 14 days later with 2.5 mg PMA in 0.1 mL acetone 3 times weekly.
[b] Compounds applied in 0.1 mL acetone 3 times weekly. NT = Not tested.
[c] Number of mice with squamous cell carcinomas are in parenthesis.
[d] Lung tumors were benign papillomas, and stomach tumors were papillomas of the forestomach.
[e] Positive control agent.
[f] Statistically significant by chi-square test compared to controls.

SOURCE: Van Duuren et al., 1979.

TABLE 8-13. SUBCUTANEOUS INJECTION OF TRICHLOROETHYLENE (30 FEMALE HA:ICR SWISS MICE PER GROUP)

Compound and dose[a]	Days on test	No. of mice with local sarcomas[b]	P value[c]
Trichloroethylene, 0.5	622	0	NS
B-propiolactone,[d] 0.3	378	24	<0.0005
Trioctanoin, 0.05 mL	631	0	-
No treatment (100 mice)	649	0	-

[a] Compound subcutaneously injected in left flank once weekly in 0.05 ml trioctanoin. Dose is mg per injection per mouse.

[b] Histopathologically characterized as fibrosarcomas.

[c] NS = Not significant. Statistical significance calculated by chi-square test compared to controls.

[d] Positive control agent.

SOURCE: Van Duuren et al., 1979.

TABLE 8-14. CARCINOGENICITY OF TRICHLOROETHYLENE BY INTRAGASTRIC FEEDING IN MALE AND FEMALE HA:ICR SWISS MICE (30 MICE PER GROUP)

Compound and dose[a]	Sex	Days on test	No. of mice with forestomach tumors[b]	P value[c]
Trichloroethylene, 0.5	M	622	1	NS
	F	622	2	NS
B-Propiolactone, 1.0	M	539	24(23)	<0.0005
	F	582	25(23)	<0.0005
Trioctanoin, 0.1 mL	M	631	0	-
	F	635	0	-
No treatment				
(100 mice)	M	649	5	-
(60 mice)	F	636	8(1)	-

[a] Dose in mg per intubation per mouse. Compounds were given once weekly in 0.1 mL trictanoin.
[b] Number of mice with squamous cell carcinoma of the forestomach is given in parentheses.
[c] NS = Not significant. Statistical significance calculated by chi-square test compared to controls.

SOURCE: Van Duuren et al., 1979.

skin relative to other organs such as liver. Administration of TCI more than once each week in the experiments with oral and subcutaneous treatment also might have more strongly challenged the animals for carcinogenicity. Dr. Van Duuren noted (written communication) that once-weekly subcutaneous injection would allow time for the injection site to heal before subsequent injections; however, more than 1 injection per week might have more effectively tested the carcinogenic potential of TCI at sites distant from the injection site. Routine histopathologic examination of a greater range of tissues and organs, both from the mice dosed orally as well as from the mice dosed by subcutaneous injection, might have provided a broader evaluation for carcinogenicity.

8.1.3 Inhalation Exposure: Rats

8.1.3.1 Henschler et al. (1980)--Henschler et al. (1980) described a study on the carcinogenic potential of TCI in Han:Wist rats. The initial age of the animals was not given. Thirty male and 30 female rats were assigned to each of three dose groups which were exposed to 0 (controls exposed to inhalation chamber air only), 100 (0.55 mg/L air), or 500 (2.8 mg/L air) ppm TCI vapor 6 hours daily, 5 days/week, for 18 months. The animals were individually caged in this study. Surviving rats were sacrificed at 36 months. All animals were subjected to necropsy and histopathologic examination.

The TCI sample, stabilized with 0.0015% triethanolamine, contained the following impurities by gas chromatography-mass spectrometry analysis: chloroform, carbon tetrachloride, 1,1,2-trichloroethane, 1,1,1-trichloroethane, and 1,2,2-tetrachloroethane, each present at less than 0.0000025% (w/w). Epoxides were not detected in this test material. Exposure levels of TCI in the inhalation chambers were monitored with direct ultraviolet spectrophotometry, and the flow rate of the TCI air mixture was adjusted to yield a threefold turnover of the total volume each hour. A description of the analytical method was provided by D. Henschler, University of Wurzburg, as follows: Exposure levels were automatically controlled to 100 or 500 ppm, as appropriate, and were continuously monitored in a gas-flow cell in an ultraviolet beam of 205 mm. The accuracy of this method was determined by the noise of the electronic equipment, and the registered band indicated ± 7% of the median at 100 ppm.

No effect of TCI on body weight gain was observed. A statistically significant ($p < 0.05$) decrease in survival of treated rats was not found (Table 8-15).

A carcinogenic effect of TCI was not evident in this study. Use of a maximum tolerated dose is not indicated in the study report, and the comparability of survival and body weight between control and treatment groups indicates that higher exposure levels of TCI possibly could have been used to provide a broader evaluation of TCI carcinogenicity.

8.1.3.2 Bell et al. (1978; audit of Industrial Bio-Test Laboratories, Inc. study)--A carcinogenicity study of TCI vapor in Charles River rats was conducted at Industrial Bio-Test Laboratories, Inc. (IBT) from 1975 to 1977, and audit findings by the Manufacturing Chemists Association (MCA) have been reported (Bell et al., 1978). The TCI product used in this study contained, on a weight percent basis, TCI, > 99%; diisobutylene, 0.023%; butylene oxide, 0.24%; ethyl acetate, 0.052%; N-methylpyrrole, 0.008%; and epichlorohydrin, 0.148%. Each of the three treatment groups plus an untreated control group consisted of 120 rats/sex. Treated animals were exposed to nominal concentrations of 100, 300, or 600 ppm (0.55, 0.165, or 0.330 mg/L air) TCI vapor 6 hours/day, 5 days/week, for 24 months. Surviving animals were sacrificed on termination of treatment. Thirty rats/sex scheduled for interim sacrifice to evaluate hematology and clinical chemistry were not given pathologic examinations.

TABLE 8-15. ESTIMATION OF PROBABILITY OF SURVIVAL OF RATS (%)[a]

Week	Group					
	Control		100 ppm		500 ppm	
	M	F	M	F	M	F
1	100.0	100.0	100.0	100.0	100.0	100.0
50	96.6	100.0	96.6	96.6	100.0	93.3
75	93.3	96.6	96.6	93.3	100.0	90.0
100	86.7	86.7	90.0	86.6	90.0	76.6
115	83.3	70.0	86.6	63.3	76.6	50.0
126	66.7	56.6	80.0	50.0	66.6	50.0
156	46.7	16.7	23.3	13.3	36.7	16.7

[a]By the Kaplan and Meier (1958) method.

SOURCE: Henschler et al., 1980.

According to the audit report by the MCA, there were noticeable deficiencies in the performance of this study. Examination of analytical data on the TCI vapor concentrations in the inhalation chambers revealed wide deviations from the nominal concentrations and high variability in the number of measurements made daily which do not allow a precise determination of the actual exposure levels. For example, histograms of measurements (Figures 8-9, 8-10, and 8-11) made during the initial 120 days of exposure show a clustering of data around the nominal concentrations; however, only 259/565, 393/623, and 389/598 readings made during the first 6 months of the study fell within ± 30% of the 100, 300, and 600 ppm nominal concentrations, respectively. Exposure data in the report by Bell et al. (1978) cover the initial 6 months of this

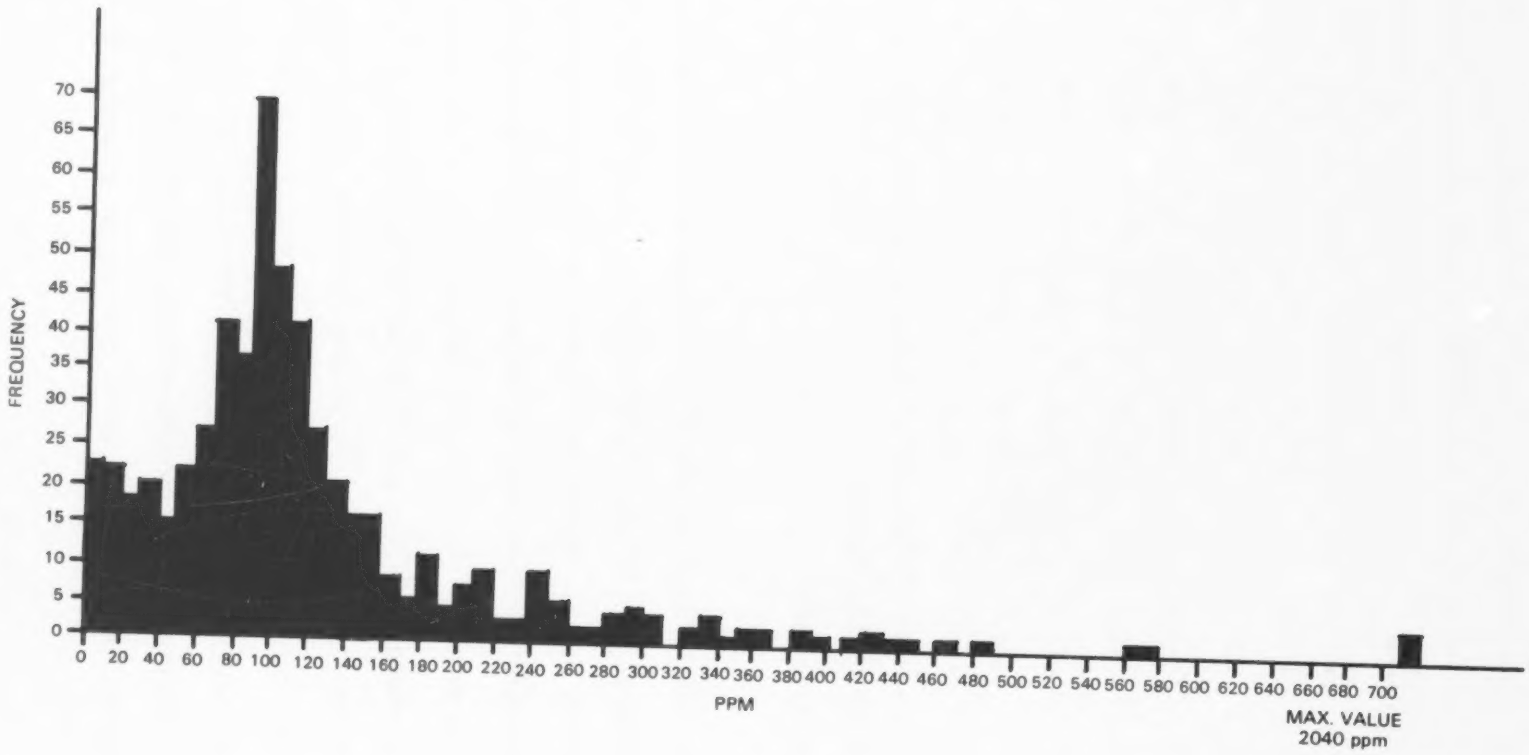

Figure 8-9. Trichloroethylene histogram of chamber concentration measurements T-1 (100 ppm).

SOURCE: Bell et al., 1978.

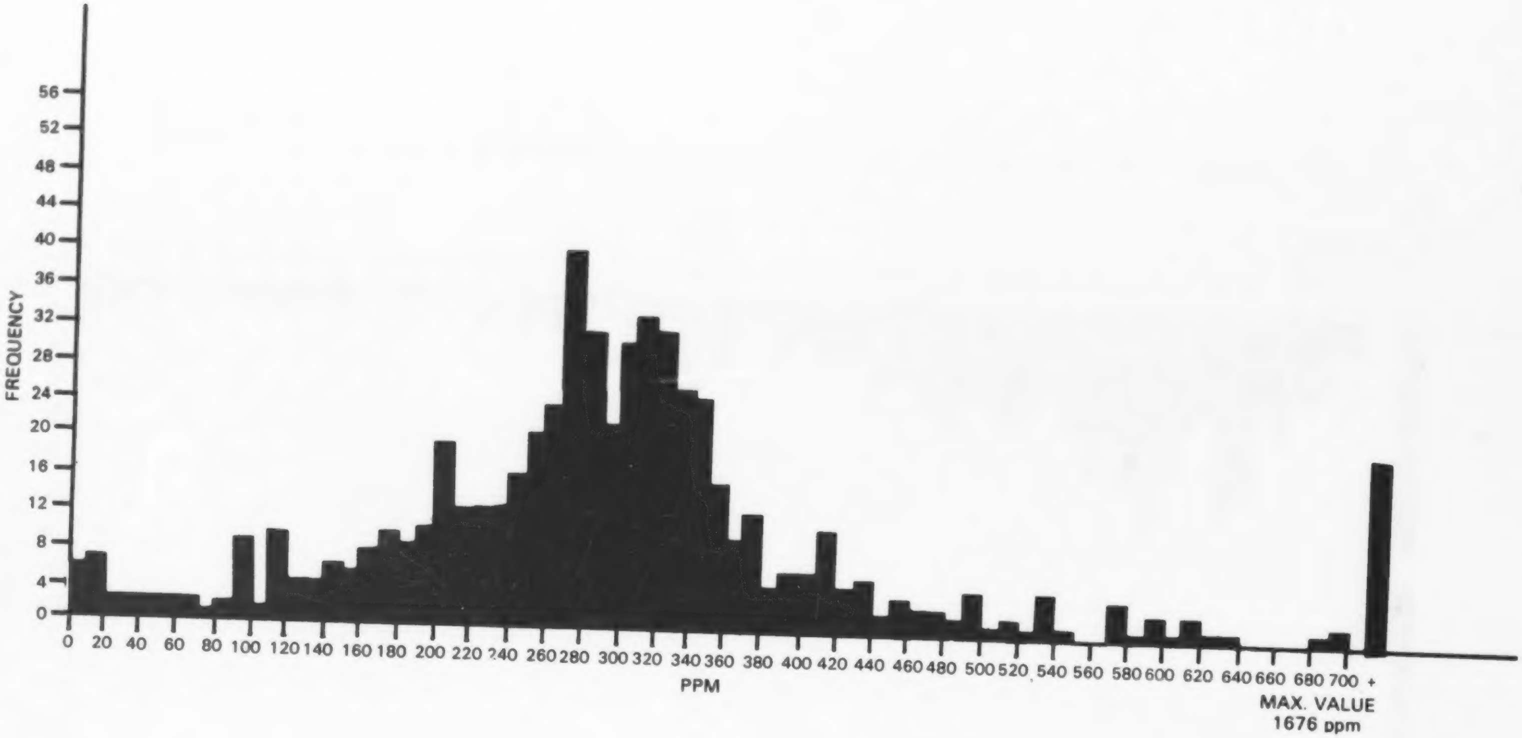

Figure 8-10. Trichloroethylene histogram of chamber concentration measurements T-1 (300 ppm).

SOURCE: Bell et al., 1978.

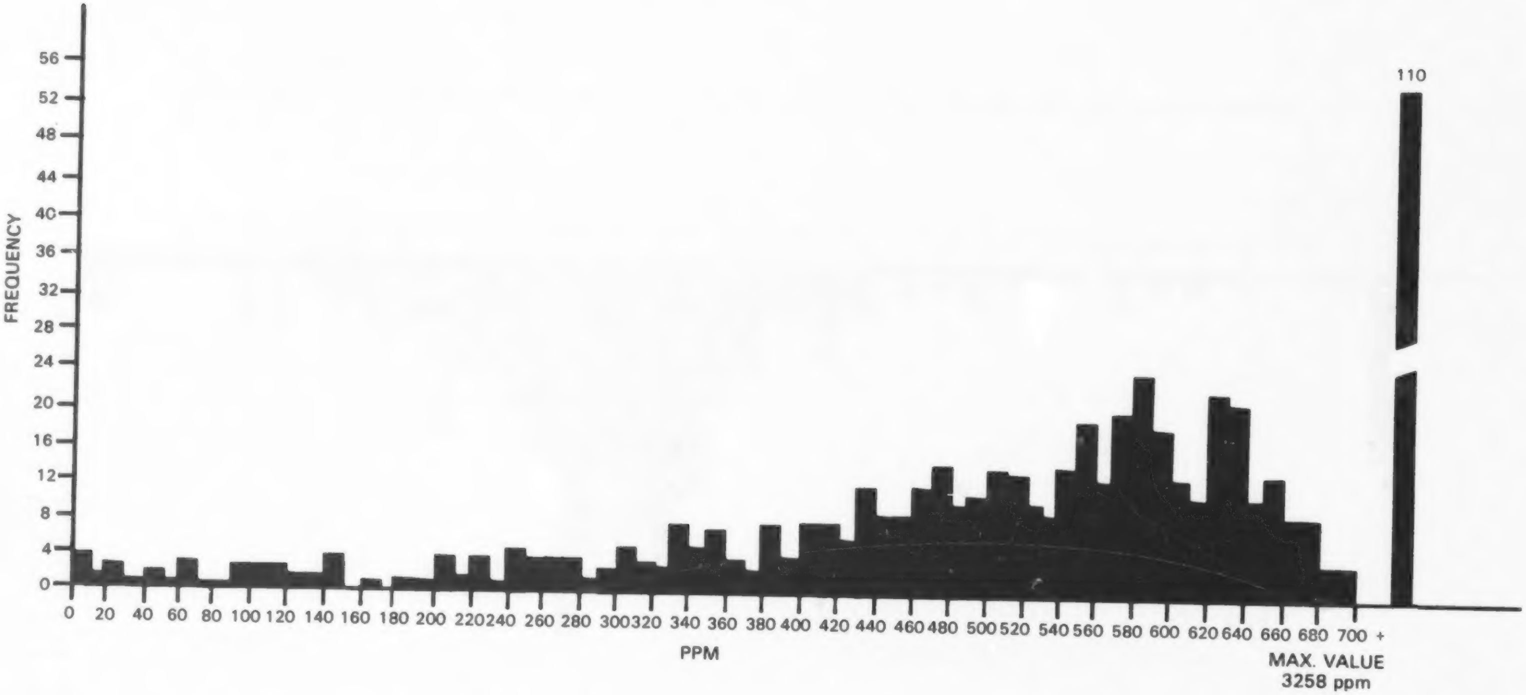

Figure 8-11. Trichloroethylene histogram of chamber concentration measurements T-3 (600 ppm).

SOURCE: Bell et al., 1978.

study, and additional information from the Chemical Manufacturers Association has been requested regarding exposure levels obtained during the final 18 months of this study.

Histopathologic reexamination of livers did not show a carcinogenic effect from exposure to TCI. Hepatocellular carcinomas were found in 3/99 control male and 3/99 low-dose male rats. Histopathologic reexamination of other tissues was not evident.

8.1.4 Inhalation Exposure: Hamsters

8.1.4.1 Henschler et al. (1980)--Henschler et al. (1980) conducted a carcinogenicity study on TCI in Syrian hamsters. The TCI sample and the experimental conditions were those used in the carcinogenicity study of TCI in Han:Wist rats by Henschler et al. (1980) discussed herein. Thirty male and 30 female hamsters per exposure group were exposed to 0 (controls exposed to inhalation chamber air only), 100, or 500 ppm TCI vapor 6 hours daily, 5 days per week, for 18 months. The initial age of the animals was not given. Terminal sacrifice was at 30 months. All animals were subjected to necropsy and histopathologic examination.

No effect of TCI on body weight gain was observed. A treatment-related effect on survival of hamsters was not found (Table 8-16); however, a marked increase in the death rate for all groups ensued after 75 weeks.

A carcinogenic effect of TCI was not evident in this study. Use of a maximum tolerated dose is not indicated in the study report, and the similarity of survival and body weight between control and treatment groups indicates that higher exposure levels of TCI possibly could have been used to provide a broader evaluation of TCI carcinogenicity.

8.1.5 Inhalation Exposure: Mice

8.1.5.1 Henschler et al. (1980)--Henschler et al. (1980) conducted a carcinogenicity study on TCI in Han: NMRI mice. The TCI sample and the experimental conditions were those used in the carcinogenicity study of TCI in Han:Wist rats by Henschler et al. (1980) discussed herein. Thirty male and 30 female mice per exposure group were exposed to 0 (controls exposed to inhalation chamber air only), 100, or 500 ppm TCI vapor 6 hours daily, 5 days per week, for 18 months. The initial age of the animals was not given. Terminal sacrifice was at 30 months. All animals were subjected to necropsy and histopathologic examination.

TABLE 8-16. ESTIMATION OF PROBABILITY OF SURVIVAL OF HAMSTERS (%)[a]

	Group					
	Control		100 ppm		500 ppm	
Week	M	F	M	F	M	F
1	100.0	100.0	100.0	100.0	100.0	100.0
50	100.0	100.0	100.0	100.0	100.0	90.0
75	100.0	83.6	100.0	89.7	100.0	76.6
100	56.7	23.3	63.3	20.7	70.0	30.0
115	40.0	6.7	26.7	-	40.0	-
126	13.3	-	6.7	-	26.7	-
156	-	-	-	-	-	-

[a] By the Kaplan and Meier (1958) method.

SOURCE: Henschler et al., 1980.

No effect on body weight gain was observed. Survival patterns described in Table 8-17 show a greater mortality in treated mice compared to controls, particularly after 50 weeks in males and 75 weeks in females. A statistically significant ($p < 0.05$) decrease in survival rates of males and females in both treatment groups compared to controls was reported. The survival patterns indicate that the TCI exposure levels used were toxic to male mice; increased mortality in treated females was particularly evident during the period when lymphoma incidence was increasing in these groups.

TABLE 8-17. ESTIMATION OF PROBABILITY OF SURVIVAL OF MICE (%)[a]

	Group					
	Control		100 ppm		500 ppm	
Week	M	F	M	F	M	F
1	100.0	100.0	100.0	100.0	100.0	100.0
50	93.3	93.3	86.7	96.6	83.3	100.0
75	83.3	83.3	63.3	73.3	56.6	82.8
100	66.7	46.7	36.6	30.0	26.7	37.9
115	33.3	33.3	16.6	13.3	13.3	10.3
126	23.3	26.7	10.0	3.3	3.3	3.4
156	-	-	-	-	-	-

[a] By the Kaplan and Meier (1958) method.

SOURCE: Henschler et al., 1980.

A carcinogenic effect of TCI was not evident in male mice in this study; however, the incidence (number affected/number examined) of malignant lymphomas in female mice was as follows: 9/29 (control), 17/30 (100 ppm), and 18/28 (500 ppm). The response in the low ($p = 0.042$) and high ($p = 0.012$) dose groups represents a statistically significant ($p < 0.05$) increase over the control incidence by the Fisher Exact Test. The time-to-tumor occurrence was shorter in treated females; however, the majority of lymphomas were apparent after treatment was terminated at 78 weeks, as illustrated in Figure 8-12. Induction of hepatocellular carcinomas by exposure to TCI was not observed in this study. Hepatocellular adenoma was diagnosed in one control and two low-dose male mice, and hepatocellular carcinoma was discovered in one control male mouse.

According to the authors, the increased incidence of malignant lymphoma in female mice could have been due to an occurrence of immunosuppression capable of enhancing the susceptibility of tumor induction by specific, mostly inborn, viruses. The 30% incidence of malignant lymphoma in matched control female mice in the Henschler et al. (1980) study is higher than the 12 to 22% (average 16%) range of lymphoma incidence determined by Luz (1977) between 1968 and 1977 for 830 untreated female NMRI-Neuherberg mice used as control groups in long-term experiments. It was the authors' impression that it was not clear whether an immunosuppressive effect would have been exerted by TCI itself or by other nonspecific influences, such as stress.

Under the conditions described in the report of their study, Sanders et al. (1982) indicated an ability of orally administered TCI to reduce the immune status in CD-1 mice; therefore, enhancement of the lymphoma incidence in the treated female mice possibly could have been related to an immunosuppressive effect from TCI treatment. Kreuger (1972) presented an argument similar to

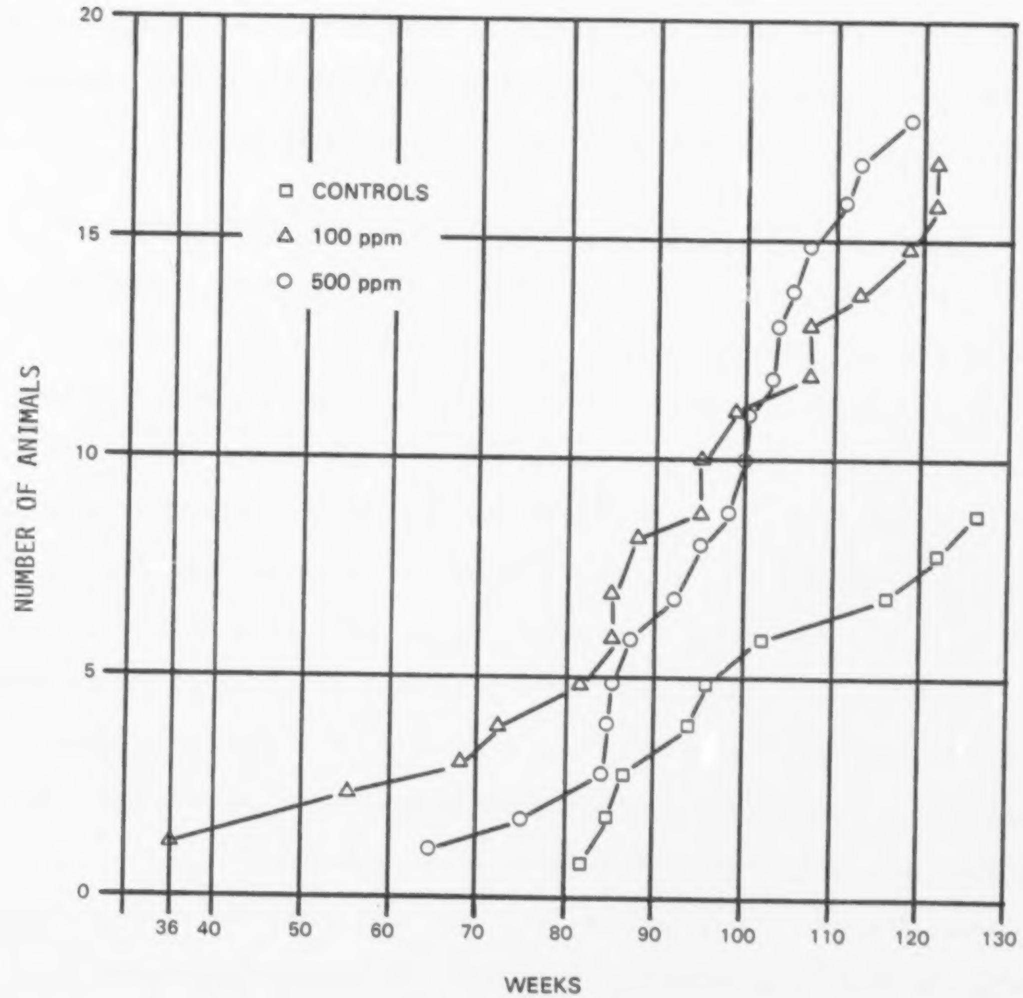

Figure 8-12. Incidence of malignant lymphomas in female mice.

SOURCE: Henschler et al., 1980.

that of Henschler et al. (1980) in which a coexistence of persistent antigenic stimulation and chronic immunosuppression is pathogenetically related to malignancy in immunocompetent tissues. Furthermore, Kreuger (1972) indicated that a significant increase in the incidence of lymphoreticular neoplasms corresponded to a significantly shortened latent period of tumor formation in several experiments cited in his report. The argument of Henschler et al. (1980), therefore, provides a possible interpretation of the results of their study.

8.1.5.2 Bell et al. (1978; audit of Industrial Bio-Test Laboratories, Inc. study)--A carcinogenicity study of TCI vapor in B6C3F1 mice was conducted at Industrial Bio-Test Laboratories, Inc. (IBT) from 1975 to 1977, and audit findings by the Manufacturing Chemists Association (MCA) have been reported (Bell et al., 1978). The TCI test sample and the experimental design were those used in the IBT study in Charles River rats discussed elsewhere herein. Each of three treatment groups (exposed to nominal levels of 100, 300, or 600 ppm TCI vapor, 6 hours daily, 5 days/week, for 24 months), as well as an untreated control group, consisted of 140 mice of each sex. Except for 40 mice/sex/group assigned to interim sacrifice for evaluation of clinical chemistry and hematology, a pathologic examination was scheduled for each animal. Surviving animals were sacrificed on termination of treatment.

Serious flaws in the conduct of this study were described in the audit report by the MCA. Wide deviations in actual exposure levels compared to nominal concentrations and high variability in the number of vapor measurements made daily, as found in laboratory records made early in the study, are described in the discussion of the IBT carcinogenicity study in rats. Furthermore, although all mice evidently were received from the same supplier, control mice appear to have been selected from a shipment obtained approximately 3 weeks earlier. Hence, the control group was not specifically matched with the treatment groups. Histopathologic reexamination of livers revealed 39 discrepancies between gross and microscopic observations, which were mostly the result of improper sectioning of liver containing tumors found grossly. Twelve mice ($\leq$ 3 per group) were identified as originally missexed during reexamination of liver specimens. The sex of nine mice ($\leq$ 2 per group) could not be determined, and for the tabulation of liver tumor data, the sex shown on laboratory records was assumed.

Statistical analysis of liver tumor data from reexamination of liver pathology was done by the Fisher Exact Test and the chi-square analysis (Table 8-18). Significant ($p < 0.05$) increases in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma/hepatocellular carcinoma combined in treated males compared to untreated males and the incidence of hepatocellular adenoma/hepatocellular carcinoma combined in treated females compared to untreated females were calculated as shown in Table 8-18. With respect to the treatment groups which were on study concurrently, statistically significant ($p < 0.05$) increases in both the number of hepatocellular carcinomas alone and hepatocellular adenomas/hepatocellular carcinomas combined in high-dose vs. either low- or mid-dose males and in hepatocellular carcinomas in high-dose vs. low-dose females were found. Increases in liver tumor incidence in high-dose compared to low-dose mice may be related to clustering of exposure level frequencies around nominal concentrations, as shown in Figures 8-9, 8-10, and 8-11 in the discussion of the IBT rat study, and increased hepatocellular carcinoma incidence was evident in B6C3F1 mice treated with TCI by gavage in the NTP (1982) and NCI (1976) bioassays discussed in this document. However, this study is weakened by the deficiencies in its conduct described above. A recently completed carcinogenicity study by Dr. C. Maltoni and

TABLE 8-18. STATISTICAL ANALYSIS OF THE INCIDENCE OF PRIMARY HEPATOCELLULAR NEOPLASMS IN CONTROL AND TRICHLOROETHYLENE TREATED B6C3F1 MICE

	Untreated controls	Males					
		T-1[a]	T-2[b]	T-3[c]	T-1 vs. T-2	T-1 vs. T-3	T-2 vs. T
Hepatocellular adenoma							
Incidence	2/99	7/95	7/100	10/97			
Percent	2.02%	7.37%	7.00%	10.31%			
Chi square		2.042	1.820	4.504*	0.032	0.214	0.329
Fisher Exact Test		0.0752	0.0873	0.0150*	0.6465	0.3223	0.2834
Hepatocellular carcinoma							
Incidence	18/99	28/95	31/100	43/97			
Percent	18.18%	29.47%	31.00%	44.33%			
Chi square		2.821	3.741	14.431*	0.006	3.930*	3.184
Fisher Exact Test		0.0463*	0.0262*	0.000063*	0.4698	0.0235*	0.0371*
Hepatocellular adenoma/ hepatocellular carcinoma combined							
Incidence	20/99	35/95	38/100	53/97			
Percent	20.20%	36.84%	38.00%	54.64%			
Chi square		5.815*	6.794*	23.408*	0.000	5.427*	4.836*
Fisher Exact Test		0.00778*	0.004407*	0.00000049*	0.4933	0.0098*	0.0138*

	Untreated controls	Females					
		T-1	T-2	T-3	T-1 vs. T-2	T-1 vs. T-3	T-2 vs. T-3
Hepatocellular adenoma							
Incidence	2/99	5/100	1/94	4/99			
Percent	2.02%	5.00%	1.06%	4.04%			
Chi square		0.572	0.002	0.172	1.364	0.000	0.719
Fisher Exact Test		0.2265	0.8672	0.3413	0.9824	0.7461	0.2007
Hepatocellular carcinoma							
Incidence	6/99	4/100	9/94	13/99			
Percent	6.06%	4.00%	9.57%	13.13%			
Chi square		0.116	0.1418	2.096	1.599	4.205*	0.303
Fisher Exact Test		0.8382	0.2604	0.0730	0.1026	0.0188*	0.2916
Hepatocellular adenoma/ hepatocellular carcinoma combined							
Incidence	8/99	9/100	10/94	17/99			
Percent	8.08%	9.00%	10.64%	17.17%			
Chi square		0.000	0.132	2.930	0.020	2.250	1.211
Fisher Exact Test		0.5083	0.3579	0.0427*	0.4427	0.0662	0.1353

*Significant at 95% level.
[a] T-1: 100 ppm
[b] T-2: 300 ppm
[c] T-3: 600

SOURCE: Bell et al., 1978.

associates can provide further evaluation of the carcinogenic potential of TCI vapor in B6C3F1 mice.

8.1.6 Other Carcinogenicity Evaluations of Trichloroethylene--Although done mainly to estimate general toxic effects of TCI, as discussed elsewhere in this document, the studies summarized in Table 8-19, except for that by Laib et al. (1978), were described by Waters et al. (1977) as studies in which carcinogenic activity for TCI was not demonstrated. However, these studies involved small groups of animals and durations of treatment and observation which were short with respect to the lifetime of the animals or, in the study by Rudali (1967), a duration which was unspecified.

Laib et al. (1979, 1978) histochemically evaluated livers from Wistar rats exposed to either vinyl chloride (VC) or TCI, by the protocol shown in Table 8-19, for focal deficiencies of nucleoside triphosphatase (ATPase) as evidence for pre-malignant hepatocellular lesions. The authors stated that Friedrich-Freksa had shown that focal deficiencies of distinct hepatocellular enzymes precede formation of hepatocellular malignancies. There was no effect in adult female rats exposed to either chemical, and ATPase-deficient foci were found in livers from newborn rats exposed to VC, but not in livers from newborn rats exposed to TCI. The authors concluded that this experiment did not indicate an oncogenic potency for TCI in hepatocytes of newborn rats. However, it is not certain whether a longer duration of exposure of newborn rats to TCI could ultimately have resulted in enzyme-deficient foci in liver.

8.1.7 Trichloroethylene Oxide

8.1.7.1 Repeated Skin Application and Subcutaneous Administration: Mice

8.1.7.1.1 Van Duuren et al. (1983). Van Duuren et al. (1983) evaluated the carcinogenicity of six epoxides of structurally related chloroalkenes by

TABLE 8-19. SUMMARY OF NEGATIVE CARCINOGENICITY DATA FOR TRICHLOROETHYLENE

Species	Number	Exposure[a]	Results	Reference
Dogs	16	Inhalation 150-750 ppm in air 20-48 hr/wk for 7-16 wk	No tumors; no deaths	Seifter, 1944
		Inhalation		
Rats	12	3,000 ppm, 27 exposures, 36 days	Three rats died; no tumors	Adams et al., 1951
Guinea pigs	11	100 ppm, 132 exposures, 85 days		
Monkeys	2	200 ppm, 148 exposures, 212 days		
Rabbits	4	200 ppm, 178 exposures, 248 days		
Cats	8	Inhalation 200 ppm, 178 exposures, 248 days	No tumors; no deaths	Monsinger and Fiorentini, 1955
Mice	28	Intragastric 0.1 mL in 40% oil solution 2/wk, unspecified treatment period	No tumors; no deaths	Rudali, 1967
Rats Guinea pigs Monkeys Rabbits Dogs	15 15 3 3 2	Inhalation 3,825 mg/m^3, 8 hr/day, 5 days/wk, 6 months 189 mg/m^3 continuous for 90 days	No tumors; no deaths	Prendergast et al., 1967
Rats	37 newborn and 2 adults per group	Inhalation 2,000 ppm, 8 hr/day, 5 days/wk, 10 weeks followed by 2 weeks without treatment	No tumorigenic potential	Laib et al., 1979, and Laib et al., 1978[b]

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[a] Surviving animals were sacrificed on approximately the final day of exposure or, in the studies by Laib et al., observation; however, the observation period is not specified in the report by Rudali (1967).

[b] Addition to summary by Waters et al. (1977).

SOURCE: Waters et al., 1977.

repeated skin application and subcutaneous injection in chronic studies with female ICR/Ha mice. The epoxides were cis-1-chloropropene oxide (cis-CPO); trans-1-chloropropene oxide (trans-CPO); cis-1,3-dichloropropene oxide (cis-DCPO); trans-1,3-dichloropropene oxide (trans-DCPO), trichloroethylene oxide (TCIO); and tetrachloroethylene oxide (PCEO). The epoxides were prepared by a modification of the method of Kline et al. (1978) to obtain pure samples. However, cis-DCPO and trans-DCPO contained 10 to 15% m-dichlorobenzene during the initial 14 months of the study, and TCIO contained 10% dichloroacetyl chloride early in the study. Pure chemicals were stored at -70°C.

Mice were 6 to 8 weeks old when the study began. Six animals were housed per cage. There were 30 mice in each experimental group, except for 100 mice in each untreated control group. Maximum tolerated doses were estimated for the chronic studies from doses not showing clinically apparent toxicity, including histopathologic examination of treatment sites and liver, in preliminary toxicity tests of 6 to 8 weeks duration.

For the chronic skin application study, test sites were shaved initially and about every 2 weeks thereafter. The epoxides were dissolved in 0.1 mL acetone for application to the dorsal skin 3 times each week for the duration of the study at the doses described in Table 8-20, except for PCEO, which, due to its instability in acetone, was applied undiluted to skin followed immediately by application of acetone. In the subcutaneous injection study, epoxides in 0.05 mL pure tricaprylin were administered at the doses shown in Table 8-21 into the left flank once weekly for the duration of the study.

Animals were observed daily and weighed bimonthly. Papillomas were characterized as skin lesions of about 1 mm in diameter which persisted more than 30 days. Each animal was necropsied, and each lesion and tumor was histopathologically examined. Routine sections examined microscopically included skin,

TABLE 8-20. CHRONIC SKIN APPLICATION IN MICE

Thirty female ICR/Ha Swiss mice per group were painted on the dorsal skin 3 times weekly with halo-epoxides at the doses indicated in 0.1 mL acetone by micropipet except where noted.

Compound	Time (days) to first tumor	Median survival time/test duration in days	No. of mice with papilloma/total papillomas	No. of mice with squamous cell carcinoma	P value[a]
cis-CPO, 10 mg	225	>432/432	14/20[b]	10	<0.0005
trans-CPO, 10 mg	345	513/574	15/20	10[c]	<0.0005
cis-DCPO, 10 mg	371	522/574	16/19	10	<0.0005
trans-DCPO, 10 mg	255	511/573	20/27[d]	17[e]	<0.0005
TCIO, 2.5 mg		526/575	0		
PCEO, 7.5 mg[f]	268	>459/459	3/39[g]	1	0.014
m-Dichlorobenzene, 3.5 mg		520/575	0		
Dichloroacetyl chloride 250 µg		>576/576	0		
dl-Diepoxybutane, 3.3 mg (positive control)	147	462/573	23/63	21[h]	<0.0005
Acetone, 0.1 mL		>576/576	0		
No treatment (100 animals)		551/580	0		

[a] P values based on number of animals with benign and malignant tumors: X^2 test with Yates' correction was used.

[b] Three were keratoacanthomas.

[c] One animal developed a fibrosarcoma and a squamous cell carcinoma.

[d] One was a histiocytoma, and one was a keratoacanthoma.

[e] One was a fibrosarcoma in the treatment area.

[f] PCEO was applied at 5 µL (7.5 mg) by Eppendorf pipet followed immediately by 0.1 mL acetone by micropipet.

[g] One was a keratoacanthoma.

[h] Two were fibrosarcomas in the treatment area.

SOURCE: Van Duuren et al., 1983.

TABLE 8-21. CHRONIC SUBCUTANEOUS INJECTION IN MICE

Thirty female ICR/Ha Swiss mice per group were given injections once weekly of halo-epoxides s.c. in the left flank at the doses indicated in 0.05 mL tricaprylin

Compound	Median survival time/test duration in days	No. of animals with local tumors	P value[a]
cis-CPO, 1 mg	482/551	1 fibrosarcoma 1 carcinoma[b] 1 leukemia	0.052
trans-CPO, 1 mg	418/556	4 fibrosarcomas 1 carcinoma	0.003
cis-DCPO, 500 μg	500/557	3 fibrosarcomas 1 anaplastic sarcoma 1 carcinoma and mammary tumor	0.003
trans-DCPO, 500 μg	498/557	5 fibrosarcomas[c]	0.003
TCIO, 500 μg	547/558	1 fibrosarcoma	NS[d]
PCEO, 500 μg	491/558	1 anaplastic sarcoma 1 leukemia	NS
m-Dichlorobenzene, 165 μg	525/560	0	
Dichloroacetyl chloride, 50 μg	>560/560	1 fibrosarcoma	NS
dl-Diepoxybutane, 330 μg (positive control)	475/553	9 fibrosarcomas[e] 2 carcinomas 1 mammary tumor	<0.0005
Tricaprylin, 0.05 mL	>524/561	0	
No treatment (100 animals)	551/580	1 fibrosarcoma	

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[a] All mice with local tumors included in calculation of p values: χ^2 test with Yates' correction was used. Each treated group was compared with the untreated control group. C-CPO was marginally significant using the 0.050 level as cutoff point.

[b] Squamous cell carcinomas.

[c] One with a focus of angiosarcoma.

[d] NS = not significant, and $p > 0.20$.

[e] One with a focus of anaplastic sarcoma.

SOURCE: Van Duuren et al., 1983.

liver, stomach, and kidney in the skin application study, and injection site tissue and liver in the subcutaneous injection study.

As shown in Tables 8-20 and 8-21, vehicle and untreated control groups were concurrently on study. Positive control groups were treated with dl-diepoxybutane. The above-mentioned impurities m-dichlorobenzene and dichloroacetylchloride were tested at the levels in which they were present in the epoxides.

Results described in Table 8-20 show no induction of skin tumors from repeated application with TCIO, and no significant ($p < 0.05$) increase in local tumors resulted from subcutaneous injection of TCIO compared to controls (Table 8-21). The positive control animals responded with development of highly significant ($p < 0.0005$, chi-square test) tumor increases in both studies (Tables 8-20 and 8-21). The investigators stated that no significant ($p < 0.05$) incidences of malignant distant tumors were observed.

As discussed by Van Duuren et al. (1983), chloroepoxides have been considered as activated carcinogenic metabolites of chloroalkenes. The negative results for carcinogenicity with TCIO in the study by Van Duuren et al. (1983) reflect the negative results for carcinogenicity with TCI reported by Van Duuren et al. (1979) using the same experimental design. Presentation of data for each epoxide tested by Van Duuren et al. (1983) is made herein to provide evidence that the relative carcinogenic activity of the six epoxides was not specifically dependent on stereochemistry (cis or trans) or rate of hydrolysis (see Figure 8-14 herein). The authors further noted that vinyl chloride epoxide has a half-life in aqueous solution similar to that of TCIO, but that the former compound has demonstrated carcinogenicity by subcutaneous injection and initiating activity in an initiation-promotion study. As indicated by the authors, the epoxides tested would be direct-acting agents which would not have to rely on metabolic activation at the sites of carcinogenic action in their study. Under the conditions of the study by Van Duuren et al. (1983), TCIO was not carcinogenic in female ICR/Ha mice. Routine histopathologic examination of additional tissues and organs might have given a broader evaluation of pathology.

8.1.8 Cell Transformation Studies

8.1.8.1 Trichloroethylene

8.1.8.1.1 Price et al. (1978). Price et al. (1978) evaluated the potential of TCI to transform Fischer rat embryo cells (F 1706, subculture 108) *in vitro*. The TCI sample, obtained from Fisher Scientific Co., was $\geq$ 99% pure and would have been stabilized with 20 ppm triethylamine or 25 ppm diisopropylamine (personal communication with Fisher Scientific Co.). Cells were grown in Eagle's minimum essential medium in Earle's salts supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 100 U penicillin, and 100 µg streptomycin per mL. Liquid TCI was diluted 1:1000 in growth medium before exposure of cells to desired TCI doses.

Doses for the transformation assays were selected as those producing minimal reduction in plating efficiency relative to medium-only control cells in preliminary tests. In the preliminary tests, triplicate cultures were exposed to 10-fold dilutions of TCI for 4 days before fixing and staining of cells and counting of colonies. Minimal reduction in plating efficiency (3 to 16%) compared to controls was produced over the range of TCI doses tested; hence, the two highest doses (1.1×10^4 and 1.1×10^3 µM) of TCI were selected for the transformation assay.

In the transformation assay, quadruplicate cultures of F1706 cells were treated at 50% confluency with the two selected concentrations of TCI. Cells were exposed to TCI for 48 hours and then washed and refed with growth medium alone. Each culture was considered a separate series. Additional cultures were exposed to the positive control agent 3-methylcholanthrene diluted in acetone (1:1000) and growth medium to a dose of 0.37 μM. Negative control cultures were grown in the presence of acetone (1:1000) in growth medium and in growth medium alone. At each subculture, one set of flasks was retained for 2 weeks without subdivision, and the other set of flasks was subdivided 1:2 each week to yield two new sets of cultures, one for the holding series without subdivision, and one for subdivision. Cell transformation was characterized by progressively growing foci composed of cells lacking contact inhibition and orientation, and by the ability of foci to grow in semi-solid agar four subcultures after treatment. The ability of transformed cells to induce local fibrosarcomas after subcutaneous injection of 1 x 10^6 cells into newborn Fischer 344 rats was also evaluated.

Transformation of cells exposed to TCI was observed, as well as the ability of transformed cells to grow in semi-solid agar and to induce local fibrosarcomas after subcutaneous injection into rats (Table 8-22). Transformation of cells exposed to the positive control agent 3-methylcholanthrene was evident, as shown in Table 8-22. Spontaneous cell transformation was not found in negative control cells (Table 8-22).

Although transformation of F1706 cells in the presence of TCI was observed by Price et al. (1978), the potency of TCI was low relative to that of the positive control chemical, 3-methylcholanthrene. The response to TCI may reflect an ability of this cell line to convert TCI to active metabolites, and further evaluation of the effect of TCI through active metabolites possibly could have been made by additional testing with an exogenous metabolic activation system and by testing the ability of the F1706 cells to produce TCI

TABLE 8-22. IN VITRO TRANSFORMATION OF FISCHER RAT EMBRYO CELL CULTURES BY TRICHLOROETHYLENE

Compound	Dose (μM)	Morphological transformation[a]	Growth in agar at P + 4[b]	Tumor incidence[c]
				No. of animals with tumors/ No. of animals inoculated
Trichloroethylene	1.1×10^4	+5 (44)	9	13/13 (48)
	1.1×10^3	+5 (44)	8	12/12 (55)
3-Methylcholanthrene	3.7×10^{-2}	+4 (37)	124	12/12 (27)
Acetone	1:1,000	(NA)	0	0/13 (82)
Medium		(NA)	0	ND

[a] Numbers represent subcultures after treatment when transformed foci were first observed. The acetone and medium control cultures were still negative at the termination of the experiment nine subcultures after treatment. Cultures were held for 2 weeks at each subculture and stained prior to screening. Numbers in parentheses represent number of days in vitro prior to observation of morphological transformation. NA = not applicable, as there was no observed transformation by subculture 9 (81 days).

[b] Numbers represent the average number of microscopic foci per three dishes. Each dish was inoculated with 50,000 cells and held for 4 weeks at 37°C in a humidified CO_2 incubator prior to staining with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium chloride.

[c] Animals inoculated subcutaneously with cells that had been treated with chemical five subcultures earlier. Numbers in parentheses represent days postinoculation to 100% tumor incidence. ND = not done.

SOURCE: Adapted from Price et al., 1978.

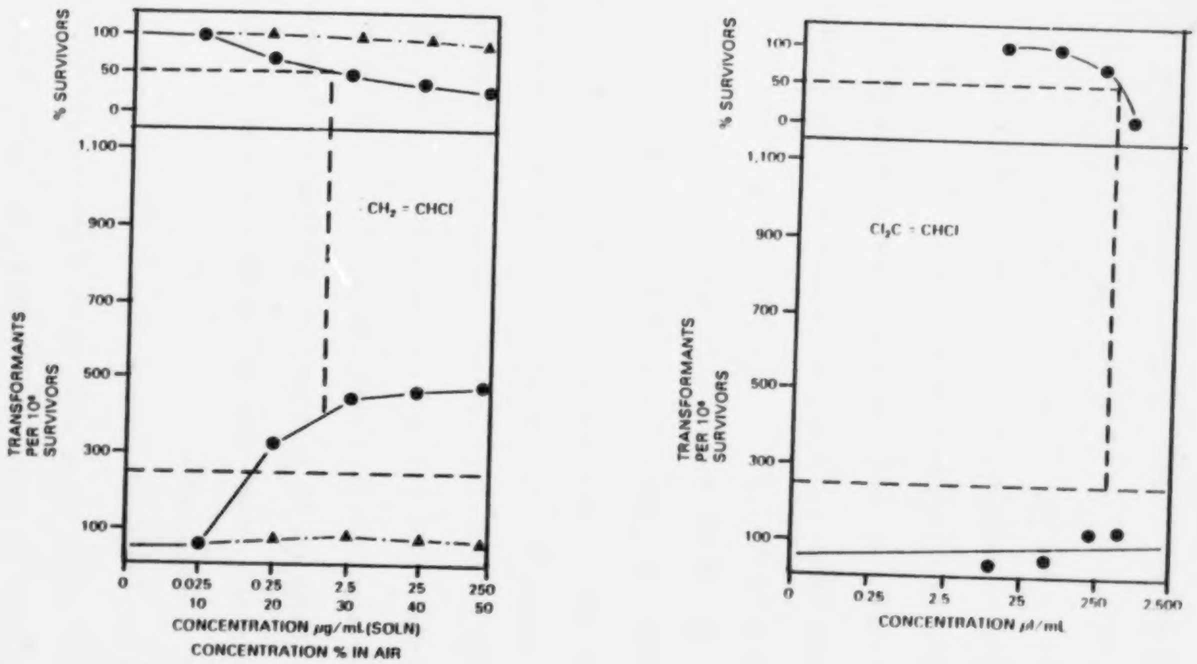
metabolites. The authors stated that the F1706 cell line, at the subculture used (108), did not require addition of murine type "C" virus and was free of any known infectious virus; however, these cells did contain genetic information of the Rauscher leukemia virus. It is not clear whether cell transformation was induced through action of the test chemicals on the host cells, their integrated oncornavirus genome, or both. On the other hand, the cell transformation test is simply an *in vitro* screening system, designed to provide at best only suggestive evidence for carcinogenicity. Additionally, cells were exposed to liquid TCI diluted in growth medium; therefore, since TCI is a volatile substance, exposure of cells to TCI as a vapor could have indicated how different exposure conditions possibly could have influenced its ability to transform F1706 cells.

8.1.8.1.2 *Styles (1979)*. Styles (1979) reported an investigation on TCI in a cell transformation system with BHK cells, using growth in semisolid agar as an endpoint, as part of a larger study (Purchase et al., 1978) done to screen chemicals for carcinogenic potential. The BHK-agar transformation assay technique used has been described by Styles (1977) and Purchase et al. (1978). In the study reported by Styles (1979), baby Syrian hamster kidney (BHK-211Cl 13) cells were exposed to five different doses of test substance *in vitro* in serum-free liquid tissue culture medium in the presence of rat liver microsomal fraction and cofactors (S-9 mix; Ames et al., 1975). The liver microsomal fraction was obtained from Sprague-Dawley rats induced with Arochlor 1254.

Cells were grown and maintained in Dulbecco's modification of Eagle's medium in an atmosphere of 20% CO_2 in air. Cells were maintained at 37°C until confluent; subsequently, the cells were trypsinized and resuspended in fresh growth medium. Resuspended cells were grown until 90% confluent for transformation assays or 100% confluent for stock. Only cells with normal morphology were used for assays. To minimize spontaneous transformation frequency, cells were obtained at low passage, grown to 90% confluency, and frozen in liquid nitrogen. Cells were thawed at 37°C in growth medium for further use.

Test compounds were dissolved in DMSO or water as appropriate. Each dose was tested in replicate assays. Cells incubated until 90% confluency were trypsinized and resuspended in Medium 199 at a concentration of 10^6 cells/mL. Resuspended cells (10^6) were incubated with test chemical and S-9 mix at 37°C for 4 hours. To evaluate compound vapors, cells (2.5×10^5) were plated and incubated until 90% confluent, at which time growth medium was replaced with medium 199 and S-9 mix. Duplicate plates were exposed to test chemical vapor at 37°C for 3 hours. After treatment, cells were centrifuged and resuspended in growth medium containing 0.3% agar. Survival after treatment was estimated by incubating 1,000 cells at 37°C for 6 to 8 days before counting colonies. Transformation was evaluated by counting colonies after cells were plated and incubated for 21 days at 37°C. The dose-response for transformation was compared to that for survival. Styles (1977) accepted a fivefold increase in transformation frequency above control values at the LC_{50} as a positive result. The spontaneous transformation frequency of BHK cells (72 experiments) in this study was 50 ± 16 per 10^6 survivors. Suitability of the soft agar medium for colony growth was checked by assays with polyomatransformed BHK-21/Cl 13 cells or Hela cells.

Data reported by Styles (1979) on unstabilized TCI (source and purity not reported) are compared with those found with vinyl chloride (VC) (ICI Mond Division; purity not reported) by Styles (1977, 1979) in Figure 8-13. Results were negative with exposure to TCI solution in DMSO added to culture medium in a dose range including levels in which toxicity was observed. Vinyl chloride



Survival and transformation of BHK cells treated with vinyl chloride gas (●——●) and vinyl chloride solution in DMSO (▲— · —▲).

Survival and transformation of BHK cells treated with unstabilized trichloroethylene solution in DMSO

Figure 8-13. Cell transformation assays on vinyl chloride and trichloroethylene in BHK-21/Cl 13 *in vitro*.

SOURCE: Styles, 1977, 1979.

was effective as a gas in producing transformation and toxicity; however, VC solution in DMSO added to culture medium was ineffective to suggest that cells were exposed to doses too low to produce an effect by this approach. Although TCI doses high enough to produce toxicity did not induce transformation, exposure of cells to TCI as a vapor could have provided a comparison of the transformation potential of TCI as a vapor and TCI in liquid solution, as was done for VC. Thus, the negative results reported in this study may, in fact, be due to its incompleteness.

The study by Purchase et al. (1978), which was done on 120 chemicals of various classes, showed that the BHK-agar transformation assay system was about 90% accurate in discriminating between compounds with demonstrated carcinogenic or noncarcinogenic activity, and was in approximately 83% agreement with results from assays done by the authors with *S. typhimurium* (TA 1535, TA 1538, TA 98, TA 100). Styles (1979) and Purchase et al. (1978) indicated, without presenting numerical data, that results were obtained in the Salmonella assays on VC in liquid solution, VC gas, and TCI in liquid solution that were similar to results found in the transformation assays. Purchase et al. (1978) also observed that metabolically activated agents transformed BHK cells more strongly in the presence of S-9 mix, thus suggesting that BHK cells have limited intrinsic metabolic capability.

8.1.8.2 Trichloroethylene Oxide

8.1.8.2.1 DiPaolo and Doniger (1982). DiPaolo and Doniger (1982) assessed the capacity of putative epoxide metabolites of six chloroalkenes, including TCI, to induce morphologic transformation in cultured Syrian hamster cells. The tested epoxides were *cis*-1-chloropropene oxide (c-CPO); *trans*-1-chloropropene oxide (t-CPO); *cis*-1,3-dichloropropene oxide (c-DCPO); *trans*-1,3-dichloropropene oxide (t-CPO), trichloroethylene oxide (TCIO); and tetrachloroethylene oxide (PCEO). TCIO and PCEO were prepared by auto-oxygenation of TCI and PCE with removal of chloride by-products by extraction with 0.5N sodium hydroxide. The other epoxides were synthesized by oxidation of the parent compounds in m-chloroperbenzoic acid. Each epoxide was provided pure, as determined by nuclear magnetic resonance analysis, by Drs. Kline and Van Duuren of New York University.

Cells for culture were obtained from Syrian hamster embryos removed from their mothers on the twelfth and thirteenth days of gestation. Primary cultures were derived by seeding 1 x 10^7 cells/dish from trypsinized embryos. Secondary cultures were made 2 to 3 days later by seeding 5 x 10^6 cells/10 mL complete medium/plate. Mass cultures were grown in modified Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum (FBS) under an atmosphere of 11% carbon dioxide.

For each plate, 300 cells from secondary cultures were plated in complete medium with 20% FBS along with 6 x 10^4 feeder cells previously irradiated as confluent monolayer cultures. After incubation for 2 days, the medium was removed, the cells were washed with phosphate-buffered saline (PBS), and the desired concentration of epoxide in PBS was added to the cells. After incubation of the cells in the presence of epoxide for 30 minutes at 37°C, the PBS was removed, the cells were washed, and fresh complete medium was added. After further incubation for 5 days, the cells were washed with PBS, fixed, and stained with Giemsa. Cell colonies $\geq$ 2 mm in diameter were counted for cloning efficiency and were examined for morphologic transformation, exhibited as criss-crossing and piled-up cells not seen in controls. Transformation frequency was calculated relative to surviving colonies and initial number of cells plated. Twelve dishes of cells were evaluated per dose.

Results of the cell transformation assay with TCIO show a weak transformation effect, with high concentrations (>1 mM) capable of producing cytotoxicity in the form of a dose-related reduction in cell survival (Table 8-23). The weak effectiveness of TCIO at high concentrations may relate to its short stability half-life of 1.3 minutes in aqueous solution (Figure 8-14). However, chemical stability in aqueous solution alone might not have been directly correlated with the ability of the six epoxides to transform cells in this study. The most stable of the six epoxides in aqueous solution, c-DCPO and t-DCPO (Figure 8-14), were the most potent inducers of cell transformation (Table 8-23), but the trans isomer was a stronger inducer than the cis. Additionally, although PCEO and c-CPO had similar stability half-lives in aqueous solutions (Figure 8-14), c-CPO was the stronger inducer of cell transformation, which, as suggested by the authors, may reflect a more limited ability of PCEO to interact with DNA. Although use of a positive control agent was not indicated for this assay, the authors mentioned that transformed colonies found in this study were similar to those induced by benzo[a]pyrene in this cell line in their laboratory.

8.1.9 Summary of Animal and Cell Transformation Studies

Increased incidences of malignant tumors in animals treated with TCI were found in carcinogenicity studies on male and female B6C3F1 mice with gavage or inhalation treatment, female Han:NMRI mice with inhalation treatment, and male Fischer 344 rats with gavage treatment. Thus, there are at least four studies which indicate that TCI-exposed rodents show elevated cancer incidence. The NCI/NTP studies show an unequivocal increase in mouse liver tumors. The other two studies just cited provide additional support to the overall body of evidence, particularly since they were carried out in different animal species or

TABLE 8-23. TRANSFORMATION AND SURVIVAL OF SYRIAN HAMSTER EMBRYO CELLS TREATED WITH DIVERSE CHLOROALKENE OXIDES[a]

Compound	Dose, mM	Percent survival	Total transformed colonies	Average transformations/ cells seeded, x 10^3
Acetone		100 (29)[b]	0	
c-CPO	0.11	110	4	1.1
	0.27	94	5	1.4
	0.55	90	6	1.7
	1.1	50	12	3.3
t-CPO	0.22	96	0	-
	0.55	78	2	0.56
	1.1	54	6	1.7
	2.2	12	1	0.26
c-DCPO	0.005	92	1	0.26
	0.01	93	3	0.83
	0.02	81	1	0.26
t-DCPO	0.01	89	8	2.2
	0.025	85	8	2.2
	0.05	44	4	1.1
TCEO	1.1	91	1	0.26
	2.5	86	1	0.26
	5.0	73	4	1.1
PCEO	0.87	98	0	-
	4.3	96	3	0.83
	8.7	100	2	0.56
	17.1	70	5	1.4

[a] Plastic dishes (60 mm) were seeded with 300 cells from a secondary culture with irradiated hamster cells (6×10^4) for a feeder, grown for 2 days, and incubated with the test chemical in PBS in the absence of growth medium for 30 min at 37°C. Cells were washed, fed again, and incubated for 7 days after which colonies were stained, counted for survival data, and scored for morphologic transformation. Twelve dishes were used for each treatment.

[b] Number in parenthesis is the absolute cloning efficiency of solvent-treated cultures.

SOURCE: DiPaolo and Doniger, 1982.

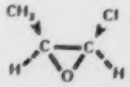
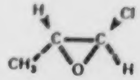
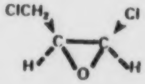
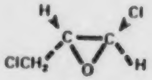
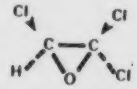
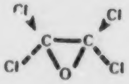
Compound	Structure	Half-Life (min)
cis-1-Chloropropene oxide (*c*-CPO)		11
trans-1-Chloropropene oxide (*t*-CPO)		4.3
cis-1,3 Dichloropropene oxide (*c*-DCPO)		290
trans-1,3-Dichloropropene oxide (*t*-DCPO)		300
Trichloroethylene oxide (TCIO)		1.3
Tetrachloroethylene oxide (PCEO)		12

Figure 8-14. Structures and stability of chloroalkene oxides. Half-life values are in minutes and were measured at pH 7.4 and 37°C as determined by Kline et al. (1978).

SOURCE: DiPaolo and Doniger, 1982.

strains. Incidences of malignant tumors were not observed to be increased in animals treated with TCI in carcinogenicity studies on female Fischer 344 rats, male and female Osborne-Mendel rats, male and female Sprague-Dawley rats, and male and female ICR/Ha Swiss mice, all with gavage treatment; male and female Charles River rats, male and female Han:Wist rats, male and female Syrian hamsters, and male Han:NMRI mice, all with inhalation treatment; female ICR/Ha Swiss mice with subcutaneous injection; and female ICR/Ha Swiss mice with topical application. Increased incidences of malignant tumors were not found in carcinogenicity studies of TCI oxide in female ICR/Ha Swiss mice with subcutaneous injection or topical application. Tumor-initiating activity was not observed in studies where either TCI or TCI oxide was applied to the skin of female ICR/Ha Swiss mice as the initiating agent. Cell transformation activity by TCI in cultured Fischer rat embryo cells and by TCI oxide in Syrian hamster embryo cells was reported. Cell transformation activity for TCI was not found in cultured baby Syrian hamster kidney cells; however, when vinyl chloride was tested under the same conditions as TCI, no transformation occurred with either compound (see complete section 8.1 for details).

8.2 DESCRIPTION AND ANALYSIS OF EPIDEMIOLOGIC STUDIES

There are currently six epidemiologic studies which relate to the issue of TCI exposure and cancer.

8.2.1 Axelson et al. (1978)

Axelson et al. (1978) found no excess mortality due to cancer in a cohort study of 518 Swedish male TCI production workers. Workers with $\geq$ 10 years of observation were identified as a subcohort. The authors did not indicate the number of workers in this subcohort, but did indicate that the subcohort included 3,643 of the 7,688 total person-years of observation in the study. Since the 1950s the TCI manufacturer had provided, free of charge, two urinary analyses per year per worker of the TCI metabolite trichloroacetic acid (TCA). Additional analyses were reported to have been provided at low cost. Using this information, the subcohort with ≥ 10 years of observation was divided into low (average reading of TCA in the urine of less than 100 mg/L) and high (average reading of TCA in the urine of more than 100 mg/L). A level of 100 mg/L of TCA in urine was reported to correspond roughly to an 8-hour time-weighted average exposure of 30 ppm TCI in the air, which is the Swedish occupational standard for TCI. The expected number of deaths was calculated by multiplying the person-years of observation by cause- and age-specific national death rates from the respective calendar years and summing the fractional age and calendar-year contributions over the entire cohort. Table 8-24 compares the expected and observed numbers of deaths for the cohort and subcohort.

There were no significant differences between the observed and the expected numbers of cancer deaths in the total cohort or in any of the subcohorts. The high exposure group, with ≥ 10 years of observation, had a risk ratio of 1.7 for cancer deaths, but this was not statistically significant. Two deaths from stomach cancer and two from leukemia occurred in the cohort; the authors did not indicate what the expected number of deaths for these sites would be, however.

Although the data presented in this study do not demonstrate that TCI is a human carcinogen, there are several limitations in the study design, or at least in the manner in which the data are reported, that would restrict any conclusions regarding this study. For example, little information is available concerning the duration of exposure of the workers. No definition of minimum exposure period seems to have been made by the authors, so that workers who were exposed for a very short period of time could have been included in the

TABLE 8-24. A COHORT STUDY OF MORTALITY AMONG MEN EXPOSED TO TRICHLOROETHYLENE IN THE 1950s AND 1960s AND FOLLOWED THROUGH DECEMBER 1975[a]

Cohort	Cause of death	Person-yrs. at observation	Observed	Expected	Risk ratio	Confidence 95% limits risk ratio
Total cohort	All deaths	7688	49	62.0	0.8	0.6 - 1.0
	Tumor deaths		11	14.5	0.8	0.4 - 1.4
Subcohort (≥ 10 years of latency time)	All deaths	3643	37	39.8	0.9	0.7 - 1.3
	Tumor deaths		9	9.5	0.9	0.4 - 1.8
- high exp.	All deaths	548	8	7.6	1.1	0.5 - 2.1
	Tumor deaths		3	1.8	1.7	0.3 - 4.9
- low exp.	All deaths	3095	29	32.2	0.9	0.6 - 1.3
	Tumor deaths		6	7.7	0.8	0.3 - 1.7

[a] Exposure categorization is based on trichloroacetic acid (TCA) in the urine, with low exposure referring to those not exceeding a concentration of 100 mg/L TCA in urine on average.

SOURCE: Adapted from Axelson et al., 1978.

study cohort. Secondly, the definition of high and low exposure for the subcohort was based on the average of TCA levels in the urine with no regard to how long the individual may have worked in the high or low exposure area. Thus, an individual may have had very low TCA levels over a short period of time, despite the fact that most of his working time was in a high-exposure area where few samples had been taken. Thirdly, the number of workers in the study (518) was relatively small for the detection of a significant risk of cancer from TCI exposure. Furthermore, it should be noted in this regard that only half of the total person-years of observation were for the group with ≥ 10 years of observation, indicating that most of the persons in the cohort had less than 10 years of observation. Thus, less than 259 people had been followed for as long as 10 years, which itself may not be a long enough observation period in which to observe an increase in cancer mortality, considering that a latency period for cancer may be as long as 20 years. Finally, no analysis was made with regard to specific tumor sites. If TCI is a carcinogen, it is more likely to act on one particular organ or tissue site than on all organ or tissue sites uniformly. It is interesting that 2 of the 11 deaths from cancer in this cohort were from leukemia (18%), whereas approximately 4% of cancer deaths among males in Sweden are leukemia deaths.

In summary, this study does not indicate that TCI is a human carcinogen; however, there are severe limitations in interpreting the data. It should also be noted that the primary author has reported in personal communications (Axelson 1980 and 1983), that the study is being updated through 1980 and that the cohort will be expanded to include 1100 workers who were exposed to TCI between 1970 and 1975.

8.2.2 Tola et al. (1980)

An ongoing cohort study on workers in Finland exposed to TCI was reported by Tola et al. (1980). According to the authors, the cohort is to be followed and analyzed every fifth year. The cohort consisted of 1,148 men and 969 women known to have been occupationally exposed to TCI at some time between 1963 and 1976. Names of workers were obtained from the biochemical laboratory of the Institute of Occupational Health in Finland, where most of the analyses for urinary trichloroacetic acid (TCA), a TCI metabolite, were done as part of the routine medical examinations for workers. Urinalyses were done by the Fujiwara reaction, which was reported to be sensitive to levels as low as 1 mg TCA/L urine. The list of names of persons whose urinary TCA levels had been measured was supplemented with the names of persons who had been reported to have TCI poisoning, according to the Occupational Disease Register of Finland, and with the names of persons who had been reported by employees to have been exposed to TCI. After confirming worker identities, the vital status and the causes of death of those who died as of November 30, 1976 were obtained from the Population Data Register of the Central Statistical Office in Finland, and cancer death information was made available by the Finnish Cancer Registry.

Urinary TCA levels for 2,004 workers were reported. The majority (91%) of the workers had TCA levels below 100 μg/L urine, which, according to Axelson et al. (1978), corresponds to an ambient exposure level of 30 ppm TCI. Furthermore, 78% of the workers had urinary TCA levels below 50 mg/L urine. An additional 80 workers registered with clinical poisoning from exposure to TCI were also part of the cohort; however, urinary TCA measurements were not available for these workers.

The observed numbers of all deaths and of cancer deaths were compared with those expected based on national mortality statistics for 1971. A similar comparison was done for a subcohort of workers exposed before 1970.

Results on the total cohort and subcohort are summarized in Table 8-25. No statistically significant ($p < 0.05$) differences between the observed and the expected numbers of deaths from cancer were found for either the total cohort or the subcohort. Tumor sites for those dying from cancer included the gall bladder, lung, breast, uterus, testis, and multiple myeloma. Two workers with more than 100 μg TCA/L urine and one worker with clinical poisoning had cancer; however, the difference of three cases from the expected number was not statistically significant ($p < 0.05$).

An excess cancer incidence among workers exposed to TCI was not found in this study. The study, however, had a number of limitations. The duration of follow-up was short. The age structure of the cohort was such that 60% of the males and 40% of the females were less than 40 years old at the end of follow-up. The possibility exists, as indicated by the authors, that some workers with low urinary TCA levels actually could have been exposed to another degreasing solvent but were included due to the use of an improper exposure test by the attending physician. The actual durations of exposure for the cohort were unknown.

TABLE 8-25. COMPARISON OF WORKERS EXPOSED TO TRICHLOROETHYLENE WITH THE TOTAL FINNISH POPULATION FOR MORTALITY FROM ALL CAUSES AND FOR CANCER MORTALITY

Person-years	All deaths Expected	All deaths Observed	Neoplasms Expected	Neoplasms Observed
Total cohort				
Males				
7,041	52.1	40.0	6.6	5.0
Females				
6,892	32.2	18.0	7.7	6.0
Subcohort (workers exposed before 1970)				
Males				
4,269	36.1	33.0	6.3	5.0
Females				
4,822	25.8	13.0	6.0	4.0

SOURCE: Adapted from Tola et al., 1980.

8.2.3 Malek et al. (1979)

Malek et al. (1979) studied the incidence of cancer among a group of 57 dry-cleaners in Prague, Czechoslovakia, who used TCI as a cleaning solvent. Exposure to TCI was verified by analysis of the urine for trichloroacetic acid. This group was reported to represent 86% of all men in Prague who had spent at least one year working in this type of service since the 1950s. The follow-up was from 5 to 50 years with 50% of the workers reported to have been followed for more than 20 years. Six cases of cancer were reported for the group, which was reported to be not significantly ($p < 0.05$) different from what would be expected. The expected number of cancer cases was not included in the authors' report, however. The primary weakness of this study is, of course, the small sample size, with the resulting weakness in the power of the study to detect a significant increase in cancer incidence above that expected.

8.2.4 Novotna et al. (1979)

Novotna et al. (1979) identified 63 histologically confirmed cases of liver cancer in the city of Prague, Czechoslovakia, for the years 1972 and 1974, and reviewed their employment histories for evidence of exposure to TCI. The study used no controls. On the basis of employment information dating back to January 1, 1957, 56 of the cases could be identified as never having been occupationally exposed to TCI. For seven cases there was no occuptional history. The fact that none of the liver cancer cases had any occupational exposure to TCI is not particularly surprising considering that in the City of Prague, which has a population of approximately one million, there were, at the time of the study, only 544 workers exposed to TCI at 63 work places. The authors indicated, however, that at one time there had been 1,000 work places where there was TCI exposure, but they did not indicate when this was.

8.2.5 Hardell et al. (1981)

Hardell et al. (1981) studied the chemical exposure histories of 169 men who had malignant lymphomas (Hodgkins and non-Hodgkins disease) and 338 controls matched by age, sex, and place of residence. The men were aged 25 to 85, and were identified from the records of the Department of Oncology, University Hospital, Umea, Sweden for the period 1974-1978. Controls werre extracted from the National Population Register. The study was specifically undertaken to examine the association between malignant lymphoma and exposure to phenoxy acids or chlorophenols, but exposure to other chemicals, including TCI, was also evaluated. Exposures were ascertained by means of a self-administered questionnaire which included questions on employment, leisure time activities, exposures to various chemicals, and drug and smoking habits.

For the analysis of solvent exposures, including TCI, the authors eliminated the matching. Seven cases and three controls were reported as having had "high-grade" exposure to TCI (exposed continuously for one week or more or exposed repeatedly to brief exposures for at least one month). When compared to 60 cases and 222 controls with no exposure to solvents, chlorophenols, or phenoxy acids, the unadjusted odds ratio, calculated by the Carcinogen Assessment Group, of being a case and having an exposure to TCI is 7.88 (95% CL, 2.30, 27.04). Without adjusting for age, this result is somewhat questionable, however. Also, the ascertainment of chemical exposure by self-administered questionnaire is of doubtful value because of the varying recall abilities of individuals. Also, it is possible that the liver cancer cases may have been exposed to TCI prior to January 1, 1957, the earliest date for which the authors had employment data. Fifteen to 17 years is not particularly long when considering the latency period for a carcinogen. Thus, the limitations of this study preclude its usefulness in evaluating the carcinogenicity of TCI, although further investigation of the results is suggested.

8.2.6 Paddle (1983)

Paddle (1983) obtained from the Mersey Regional Cancer Registry in England a list of all the liver cancer cases for the period 1951-77. Information on the cases included name, address at registration, and dates of birth and registration. This list was then reduced to those who had both primary liver cancer and an address near Runcorn, which is the location of an Imperial Chemical Industries (ICI) plant that has manufactured trichloroethylene since 1909. This resulting list of 95 was then compared with a list of the "tens of thousands" of employees of the Runcorn plant who had worked during the period 1934-76. None of the 95 primary liver cancer cases who had lived near Runcorn were found to have worked in the ICI plant. As a further check, the files in the company's computerized medical records system were searched for records of past employees dying with liver cancer as the underlying cause. One liver cancer death was found for the Runcorn plant and one liver cancer was found for a neighboring plant. The cancer registry records, however, showed that the first of these had suffered a primary cancer of the esophagus with a secondary liver tumor; the other patient had had multiple secondary deposits from an unknown primary tumor.

There are several deficiencies in this study. In addition, there are limitations in the author's description which make the study difficult to evaluate. One problem is that the author chose only those individuals from the cancer registry who had addresses near Runcorn. Obviously, had a worker lived farther away or moved from Runcorn following employment at the ICI plant, he would not have been included in the study. Another problem is that the author provides no indication as to the likelihood of finding an ICI worker with TCI exposure among the 95 primary liver cancer cases. Although the author reported that the list of 95 liver cancer cases was compared with a list of "tens of thousands" of employees at the ICI plant at Runcorn, he also indicated in his report that only about "1,000 or so" had TCI exposure. No controls were used in the study; thus there is not a good basis for a statistical comparison. Lastly, for those workers with TCI exposure, it is not known how long they had been exposed, or how recent their exposures were.

8.3 RISK ESTIMATES FROM ANIMAL DATA

The evidence for the carcinogenicity of TCI reviewed in this document consists primarily of positive mouse studies (NCI, 1976; NTP, 1982; Henschler et al., 1980) and one marginally positive rat study (NTP, 1982). These positive animal studies reported an incidence of excess tumors of several types. Additional supporting evidence for the carcinogenicity of TCI includes the fact that TCI has been reported to be weakly mutagenic in numerous test systems and to have a low order of DNA binding. A metabolic intermediate, trichloroethylene oxide, has been reported as positive in cell transformation studies; there are no known differences among species with regard to metabolic pathways or metabolic profiles. The NCI and NTP studies, which show an unequivocal increase in liver tumors in B6C3F1 mice, serve as the basis for cancer risk quantification in this document.

The evidence for the carcinogenicity of TCI has an uncertain classification depending upon the differing scientific interpretations of the relevance of mouse liver tumors (hepatocellular carcinomas) to human cancer risk. However, the more conservative public health view would regard TCI as a probable human carcinogen. It is important to note that the quantitative estimations of the impact of TCI as a carcinogen are made independently of the overall weight of evidence for the carcinogenicity of TCI. The calculations are made only under the presumption that TCI is a human carcinogen.

8.3.1 Selection of Animal Data Sets

For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, may be available. A choice can be made, when appropriate, as to which of these data sets to use in the mathematical extrapolation model. It may also be appropriate to correct for differences in metabolism between species and for different routes of administration.

For TCI, the data from the NTP (1982) and NCI (1976) oral gavage lifetime studies in male and female mice, showing a significant dose-related excess of tumors (hepatocellular carcinomas), have been used. Tumors were found at both the "high" dose (approximately the maximum tolerable dose) and the "low" dose (one-half the maximum tolerable dose). Table 8-26 gives the time-weighted average dosage and tumor incidence in male and female mice for the NCI and NTP studies. TCI in corn oil vehicle was given once a day by gavage (vehicle only was given to controls); in the NTP study, 0.5 mL of oil was used to dose B6C3F1 mice by gastric intubation 5 days/week, starting at 8 weeks of age, continuing for 103 weeks, and sacrificing at 112 and 115 weeks. In the NCI study, about 0.3 mL of corn oil was used, varying with body weight, to dose B6C3F1 mice by gavage 5 days/week, starting at 5 weeks of age, continuing for 78 weeks, and sacrificing after 90 weeks (see Section 8.2.1 for details of these studies). The time-weighted average gavage doses for both studies are given in Table 8-26.

A dose-response relationship for both sexes is apparent for each of the two doses used in the NCI study. It should be noted that the chronic daily gavage doses used in the NTP and NCI studies are in the range reported for acute oral toxicity of TCI, LD_{10} to LD_{50}, in other strains of mice (see Chapter 5). However, animal survival in the NCI lifetime study (1.5 year) was 44% for high-dosed males, 72% for low-dosed males, and 84% to 89% for females.

TABLE 8-26. INCIDENCE OF HEPATOCELLULAR CARCINOMAS IN MALE AND FEMALE MICE (B6C3F1) IN THE NTP (1982) AND NCI (1976) GAVAGE STUDIES

Study	Sex	Average terminal weight (g)	Time-weighted average gavage dose[a] mg/kg/day	mg/animal/day	Incidence	
NTP	M	40	0	0	8/48	(17%)
			1,000	40	30/50	(60%)
	F	35	0		2/48	(4%)
			1,000	35	13/49	(27%)
NCI	M	33	0	0	1/20	(5%)
			1,169	38.6	26/50	(52%)
			2,339	77.2	31/48	(65%)
	F	26	0		0/20	(0%)
			869	22.6	4/50	(8%)
			1,739	45.2	11/47	(23%)

[a] $$\frac{\text{Sum (dose in mg/kg x no. of days at that dose)}}{\text{Sum (no. of days receiving any dose)}}$$

Similarly, in the 2-year NTP study, 32% of the males and 46% of the females survived chronic administration of the single daily dose given, an amount which approximated the low dose of the NCI study. Presumably, TCI is subjected to elimination processes during the 24-hour interval between doses and on weekends, and hence the chronic LD may be much higher than the acute LD, particularly when the half-life is very short. Prout et al. (1984) estimated the T 1/2 of TCI in B6C3F1 mice as only 1.75 hour, while Green and Prout (1984) demonstrated that chronic daily oral dosing of TCI (1,000 mg/kg/day) in mice (180 days) produces a consistent daily amount of urinary metabolites, indicating that accumulation of TCI with chronic daily dosing does not occur. Prout et al. (1984) and Green and Prout (1984) studied the metabolism and kinetics of TCI in B6C3F1 mice given TCI by gavage in corn oil at doses of 10 to 2,000 mg/kg, i.e., including the conditions of the NTP and NCI bioassays.

8.3.2 Interspecies Dose Conversion

8.3.2.1 General Considerations--Carcinogenesis is a complex process, not entirely understood, and no observational evidence exists to validate satisfactorily the extrapolated predictions of carcinogenesis from animal models to humans. Thus, there is no scientific basis per se for choosing one extrapolation method over another in extrapolation of the carcinogenic response. However, in extrapolating the dose-carcinogenic response relationships of laboratory animals to humans, the doses used in the bioassays must be adjusted in some way to allow for such differences as size and metabolic rates. Therefore, other biological information, particularly interspecies data, is examined for TCI, since physiologic, biochemical, and toxicologic responses other than cancer may provide information as to what factors might be considered and what method is generally appropriate for the extrapolation from laboratory animals to humans for this chemical.

The major components requiring consideration in determining an appropriate extrapolation base for scaling carcinogenicity data from laboratory animals to humans are: 1) toxicologic data, 2) metabolism and kinetics, and 3) covalent binding. The biological basis for extrapolation of dose-carcinogenic response relationships has been outlined and discussed previously by Davidson (1984) and Parker and Davidson (1984).

8.3.2.1.1 Toxicologic Data. The acute lethal concentrations or LD_{50}s for inhalation and oral exposures to TCI are remarkably similar across species (mice, rats, dogs, and humans), presumably due to an equally effective concentration leading to central nervous system depression, resulting in death (Smith, 1966). Klassen and Plaa (1966, 1967) and Tucker et al. (1982) have investigated other toxicologic end points (liver and kidney enzyme activity changes and other modalities of liver and kidney damage), but these observations have not been extended across more than one to two species (see Chapter 5). Hepatotoxicity of TCI has been shown to be directly related to the extent of the metabolism of the parent compound (Buben and O'Flaherty, 1985).

8.3.2.1.2 Metabolism and Kinetics. The carcinogenic potential of TCI is generally considered to reside in cellular "reactive" intermediate metabolites of TCI (e.g., TCI oxide, chloral, dichloroacetyl chloride, formyl chloride) rather than in the intact molecule itself. These metabolites have been postulated to be responsible, for example, for the hepatotoxicity of TCI (as expressed by cellular morphological and functional changes) and its cellular genotoxicity (as expressed by DNA binding), although the primary mechanism (genotoxic or nongenotoxic) for TCI's carcinogenic potential in mice has not been satisfactorily established. The metabolism of TCI has been relatively extensively studied in three species: mice, rats, and humans. Limited pharmacokinetic data are also available. (See Chapter 4 for a detailed review of the metabolism and pharmacokinetics of TCI.) The pertinent metabolism and pharmacokinetic information which might be applied to extrapolation of TCI dose-carcinogenic response relationships from laboratory animals to humans is discussed in the sections that follow.

In mice, rats, and humans, the principal end metabolites of TCI are common: trichloroacetic acid (TCA), trichloroethanol (TCE) and TCE-glucuronide, and the precursor metabolite, chloral. In addition, TCI oxide has been demonstrated to be formed by both mouse and rat hepatic microsomes in vitro (Miller and Guengerich, 1982). Minor metabolites, including carbon dioxide, dichloroacetic acid, oxalic acid, and N-(hydroxyacetyl)-aminoethanol (HAEE), have also been identified for each of these species (see Chapter 4). Green and Prout (1984) and Dekant et al. (1984) made comparative studies of the biotransformation of TCI in rats and mice and failed to detect any major species or strain difference in metabolism. Thus, there is persuasive experimental evidence that the metabolic pathways for TCI are qualitatively similar in mice, rats and humans.

The relative magnitude of metabolism of TCI across species, as an index of cellular damage from "reactive" metabolites, provides information for determining a "mouse to man" extrapolation scaling factor.

Four types of studies have been done to investigate dose-metabolism relationships: 1) single-dose studies across species, using ^{14}C-TCI and urinary radioactivity and/or other methods as measures of metabolism, 2) kinetic models based on inhalation kinetics, 3) comparative balance studies between rat and mouse with ^{14}C-TCI at one or two dose levels, and 4) multiple dose level studies in rats and/or mice. Comparative balance studies, which determine the metabolic fate and disposition of TCI (amount metabolized, amount excreted unchanged, etc.), have been carried out after oral dosing and after inhalation exposures in both rats and mice.

8.3.2.1.2.1 Oral Studies. The balance studies in which TCI dosing was by the oral route are the most pertinent to the conditions of the NTP and NCI bioassays. Prout et al. (1984) administered ^{14}C-TCI in corn oil in the form of single intragastric doses of 10, 500, 1,000, and 2,000 mg/kg to male Osborne-Mendel and Wistar-derived rats, and to male B6C3F1 and Swiss mice. Exhaled breath, urine, feces, and carcass were analyzed for ^{14}C-TCI radioactivity (and unchanged TCI) for up to 72 hours after dosing, and expressed as a percent of dose administered. The results of these studies, as calculated and re-expressed from the data of Prout et al., are given in Table 8-27. Regarding these data, several general observations can be made comparing the metabolism of TCI in the rat and mouse.

As indicated in Table 8-27, the recovery, from 91% to 98%, of ^{14}C-radioactivity in this experiment indicates that virtually complete gastrointestinal absorption of TCI occurred for all doses in both rats and mice. Peak blood levels occurred at about 1 hour in mice and at 3.5 hours in rats. Some 80% to 90% of the TCI was excreted, either in the form of metabolites or unchanged in exhaled breath, within the first 24 hours.

There appears to have been no rat strain difference or mouse strain difference in the disposition of TCI.

For mice, oral doses of less than 1,000 mg/kg were virtually completely metabolized. Significant amounts of unchanged TCI appeared in exhaled air at doses of 1,000 mg/kg (18%) and 2,000 mg/kg (14%), indicating saturation of metabolism beginning to occur at doses approaching 1,000 mg/kg. For rats, oral doses of less than 500 mg/kg were virtually completely metabolized, with

TABLE 8-27. DISPOSITION OF ^{14}C-TCI 72 HOURS AFTER SINGLE ORAL DOSES TO MALE OSBORNE-MENDEL AND WISTAR-DERIVED RATS AND TO MALE B6C3F1 AND SWISS MICE

	Mice (mean of 4)				Rats (mean of 4)		
Dose (mg/kg)	Dose/animal[a] (mg)	Metabolized (mg equivalent)	Exhaled (mg equivalent)	Dose (mg/kg)	Dose/animal[a] (mg)	Metabolized (mg equivalent)	Exhaled (mg equivalent)
10	0.30	0.28	0.01	10	2.0	1.93	0.03
500	15.0	13.73	0.90	500	100.0	55.40	42.70
1000	30.0	23.28	5.25	1000	200.0	79.00	112.40
2000	60.0	46.90	8.16	2000	400.0	80.40	311.20

[a] Based on experimental weight of animals: rats, average 200 g; mice, 30 g.

SOURCE: Calculated from the data of Prout et al., 1984.

saturation occurring at about this dosage level. These observations of saturation of metabolism are in accord with those of Filser and Bolt (1979) and of Andersen et al. (1980) who also observed saturation of metabolism in the rat during inhalation exposure.

For oral doses of 10 to 2,000 mg/kg (covering the range used in the NTP and NCI mouse bioassays, Table 8-26), the ratio of the amount metabolized in the rat versus the amount metabolized in the mouse was as follows:

Dose (mg/kg)	Ratio: Rat/mouse (mg metabolized/animal)
10	(1.93/0.28) = 6.89
500	(55.40/13.73) = 4.03
1000	(79.00/23.28) = 3.39
2000	(80.4/46.90) = 1.71

At the highest dose, the ratio of the amount metabolized in the rat versus the mouse is smaller than it is at lower doses, probably due to saturation of metabolism in the rat. At the lowest dose, closer to 100% of the assimilated dose is metabolized in both rats and mice. These data indicate that, for the dose range used in the cancer bioassays, the metabolism and, hence, the fractional TCI doses metabolized for rats and mice are more consistent with a $W^{2/3}$ base (surface area) than a $W^{1.0}$ base (mg/kg).

Green and Prout (1984) orally dosed rats and mice subchronically for 180 days with TCI in corn oil at a level of 1,000 mg/kg/day. The relative proportions of urinary metabolites (TCA and TCE-glucuronide) were unaffected by strain differences. The amount of the daily dose metabolized stayed constant (Table 8-28), although the ratio of TCA to TCE-glucuronide increased. These observations indicate that 1) even at a high dosage level, TCI does not accumulate significantly with daily dosing, i.e., the total body half-life is suf-

TABLE 8-28. METABOLISM OF TCI IN B6C3F1 MICE: EFFECT OF CHRONIC DOSING (1,000 mg/kg/day[a])

Day	Metabolized (mg equivalent)	Expired unchanged (mg equivalent)
	Chronic	
1	18.27	5.28
10	22.50	4.08
180	15.75	7.11
	Single-dose controls	
1	18.27	5.28
10	19.35	4.62
180	16.86	3.75

[a] Based on experimental weight of mice averaging 30 g, the daily dose per mouse equals 30 mg in 0.5 corn oil.

SOURCE: Recalculated from the data of Green and Prout, 1984, based on an after-dosing collection period of 24 hours.

ficiently short (1 to 2 hours) so that each daily dose is cleared (metabolized, exhaled, etc.) within the 24-hour dosage interval; and 2) the increased ratio of TCA to TCE-glucuronide suggests a possible adaptation of metabolic pathways, which may occur simply because TCA has a longer half-time in the body than TCE. Prout et al. (1984) estimated the T 1/2 for TCI in mice at 1.75 hours; therefore, 98% elimination occurs in 10.5 hours (T 1/2 x 6), and for some 14 hours of each dose interval (24 hours), exposure to the highly reactive intermediates formed from TCI is practically nil. For the rat or human with longer half-times of elimination, proportionally less time free from exposure to the active chemical species is available (T 1/2 = 4 to 5 hours for humans).

Dekant et al. (1984), using balance studies, compared the metabolism of TCI in female rats (Wistar) and female mice (NMRI). ^{14}C-TCI was administered as a single dose by gavage in corn oil at 200 mg/kg. Radioactivity was determined in urine, feces, carcass, and exhaled air for 72 hours post-administration. Total recovery of radioactivity was 93% to 98%, and a virtually complete oral absorption is indicated by the results shown in Table 8-29. For this single dose, the rats excreted 52% of the TCI dose through the lungs unchanged, while the mice excreted 11% of the dose unchanged. The ratio of the amount of TCI metabolized by the rat versus that metabolized by the mouse was as follows:

Dose (mg/kg)	Ratio: Rat/mouse (mg metabolized/animal)
200	(23.04/4.56) = 5.05

The comparative surface area ratio of the two species, as calculated from their experimental body weights, is $(240/24.4)^{0.67} = 4.46$. At this dose, the amounts of TCI metabolized by the rat and the mouse are closely proportional to their relative surface areas.

TABLE 8-29. DISPOSITION OF ^{14}C-TCI RADIOACTIVITY FOR 72 HOURS AFTER SINGLE ORAL DOSE (200 mg/kg) TO RATS AND MICE (NMRI)

	Mice (av. of 3)	Rats (av. of 2)[a]
	Absolute dose 5.1 mg/animal	Absolute dose 48 mg/animal
	mg equivalent per animal	mg equivalent per animal
Expired TCI		
Unchanged	0.56 (11.0%)	24.96 (52.0%)
Metabolized		
$^{14}CO_2$	0.31	0.91
Urine	3.89	19.78
Feces	0.25	0.86
Carcass	0.10	1.39
Washes	0.01	0.10
	4.56 (89.4%)	23.04 (48.0%)
Total	5.12	48.0

[a] Based on experimental weight of animals: female rats, 240 g; female mice, 25.5 g.

SOURCE: Recalculated from the data of Dekant et al., 1984.

Buben and O'Flaherty (1985) recently examined the extent of TCI metabolism in male mice during subchronic administration by gavage in corn oil vehicle given for 6 weeks (5 days/week; conditions similar to those of the TCI carcinogenicity bioassays). While the strain was Swiss-Cox and not B6C3F1, which was used in the NTP and NCI bioassays, the results in this study are probably relevant and generally applicable. A dose-amount metabolized curve was established with doses of 0, 100, 200, 400, 800, 1,600, 2,400, and 3,200 mg/kg/day. Metabolism was measured by urinary metabolites excreted per day (24-hour urine collections). TCA (15% to 30%), TCE, and TCE-glucuronide (70% to 85%), as determined by gas chromatography, were found to be the principal metabolites; oxalic acid was not found to be a significant metabolite. Metabolites in feces represented less than 5% of the amount in urine. No significant day-to-day variability nor week-to-week trends were found in the urinary metabolites excreted. Presumably, because of the relatively short half-life of TCI (1.75 hours), urinary metabolites did not accumulate over several days with chronic dosage.

Figure 4-8 (in Chapter 4) shows the biphasic-appearing relationship observed between metabolism (amount of urinary metabolites) and TCI dose. The initial portion of the curve, from 0 to 1,600 mg/kg TCI, is linear; the second portion shows relatively abrupt plateauing, with an evident saturation of metabolism. In the linear portion of the curve, from 0 to 1,600 mg/kg dose, 27.5% of the dose was converted into urinary metabolites; above 1,600 mg/kg, the fraction of each dose metabolized decreased. For comparison, Green and Prout (1984) orally dosed mice daily for 6 months with TCI in corn oil at a level of 1,000 mg/kg/day, and found that 525-mg equivalents of TCI/kg body weight were metabolized per day (calculated from data in Table 8-28). Buben and O'Flaherty (Figure 4-8) observed, for this dose of TCI, a metabolism of 318 mg urinary metabolites/kg body weight. The data of Green and Prout were obtained with ^{14}C-TCI and provide a measure of total metabolism. It is likely that Buben and O'Flaherty's measurements of the urinary metabolites underestimated the total metabolism, since the measurements did not include TCI metabolism to carbon dioxide, metabolites in feces and bound to cellular macromolecules, and other metabolites in urine in addition to TCA, TCE, and TCE-glucuronide. The molecular weight of TCI is 131.4, that of TCA is 163.4, and that of TCE is 149.4. If an adjustment is made on the basis of 1 μmol TCI being metabolized to 1 μmol of TCA or TCE, and the mg equivalent represented by urinary metabolites is 272 mg/kg body weight. In comparison to the findings of Green and Prout, this represents an underestimation of about 50%. Such a comparison is in accord with the observations of Green and Prout that about 53% to 75% of the orally administered TCI at 1,000 mg/kg/day was metabolized (Table 8-28). Buben and O'Flaherty, on the other hand, observed a TCI metabolism fraction of 27.8%.

An important observation can be made on the basis of the data of Buben and O'Flaherty (Figure 4-8) and Prout et al. (Table 8-27), seen in relation to the NTP and NCI bioassay data. The NTP average gavage assay dose (1,000 mg/kg/day) is within the linear portion of the dose-amount metabolized curve of Buben and O'Flaherty (Figure 4-8). However, the saturation of metabolism is approached in the "high" doses (1,739 and 2,339 mg/kg/day) of the NCI bioassay and a lower percentage of the administered dose is metabolized at these doses. Assuming that Swiss-Cox mice accurately represent the metabolism characteristics of B6C3F1 mice, the metabolism (or fractional dose metabolized) contributing to a carcinogenic response can be estimated from Figure 4-8 in the following manner:

		Gavage dose (mg/kg/day)	Urinary metabolites from Figure 4-8 (mg metabolites/kg/day)	Percent increase of metabolites with dose
NTP	Males:	1000	318	
	Females:	1000	318	--
NCI	Males:	1169	372	
		2339	625	68
	Females:	869	276	
		1739	540	96

Thus, with a twofold increase of TCI dosage to male mice of the NCI study, metabolism increased by only 68%. This observation is consistent with the failure to observe a greater dose-related tumor increase in male mice in the NCI bioassay (Table 8-26).

Buben and O'Flaherty demonstrated that certain indices of TCI hepatotoxicity (increased liver weight and decreased liver glucose-6-phosphatase activity) correlated well with the amount of TCI metabolized by mice. The degree of hepatotoxicity (as measured by each of these toxicity parameters) when plotted against urinary metabolite excretion was linear, suggesting that the hepatotoxicity of TCI in mice is directly related to the metabolism of TCI.

8.3.2.1.2.2 Inhalation Studies. Stott et al. (1982) exposed rats and mice to 10 and 600 ppm of ^{14}C-TCI for 6 hours and estimated the amount metabolized by collecting the radioactivity excreted in urine, feces, expired air, etc., and by determining TCI in expired air for 50 hours post-exposure. Tables 8-30 and 8-31 summarize their results. The following general observations can be made on the basis of these data:

For mice exposed to TCI for 6 hours at 10 ppm and 600 ppm, virtually all of the total TCI uptake was metabolized. At 600 ppm exposure for 6 hours, all

TABLE 8-30. DISPOSITION OF ^{14}C-TCI 50 HOURS AFTER INHALATION EXPOSURE OF MALE B6C3F1 MICE

	10 ppm for 6 hr		600 ppm for 6 hr	
	mg-eq/kg	mg/animal[a]	mg-eq/kg	mg/animal
TCI expired	0.08 (0.8%)	0.003	10.01 (2.4%)	0.35
Total body dose	10.31	0.36	412.30	14.43
Total metabolized	10.24 (99.2%)	0.36	402.32 (97.6%)	14.0

[a] Based on 35 g mouse; calculated from data.

SOURCE: Stott et al., 1982.

TABLE 8-31. DISPOSITION OF ^{14}C-TCI 50 HOURS AFTER INHALATION EXPOSURE OF MALE OSBORNE-MENDEL RATS

	10 ppm for 6 hr		600 ppm for 6 hr	
	mg-eq/kg	mg/animal[a]	mg-eq/kg	mg/animal
TCI expired	0.10 (2.1%)	0.03	29.83 (21.1%)	7.46
Total body dose	4.70	1.18	141.24	35.31
Total metabolized	4.60 (97.8%)	1.15	111.29 (78.9%)	27.82

[a] Based on 250 g rat; calculated from data.

SOURCE: Stott et al., 1982.

of the assimilated dose metabolized was equivalent to that of an oral dose to mice of 500 mg/kg, and was approximately two- to fourfold less than the body metabolic load in the NTP and NCI mouse bioassays. For rats as well, exposure to 10 ppm resulted in the metabolism of virtually all of the TCI pulmonary uptake; however, at 600 ppm some of the total assimilated dose was exhaled unchanged (21%), indicating nonlinear kinetics and suggesting the approach of saturation of metabolism at this exposure concentration. At 600 ppm exposure of rats for 6 hours, the total body metabolic load roughly approximated that from an oral dose of 500 mg/kg when 42% of the dose was exhaled unchanged. These observations of approaching saturation of metabolism in rats at 500 ppm are in accord with those of Andersen et al. (1980), who estimated saturation at about 1,000 ppm and a V_{max} (maximum rate of the saturable process) of 28 mg/kg/hour, and are considerably higher than the observations of Filser and Bolt (1979), who estimated saturation at about 65 ppm but a comparable V_{max} of 28 mg/kg/hour. From the data of Stott et al. (1982), the V_{max} can be estimated as 19 mg/kg/hour.

The ratios of the amount of TCI metabolized by the rat versus the mouse at 10 and 600 ppm (Tables 8-30 and 8-31) are as follows:

Exposure ppm, 6 hours	Ratio: Rat/mouse
10	(1.15/0.36) = 3.19
600	(27.82/14.0) = 1.99

Therefore, on the basis of comparative surface area $(250\ g/35\ g)^{0.67} = 3.7$, the ratio of the amounts metabolized by the rat versus the mouse approximates their relative surface areas at the lower dose. At the higher dose, the ratio of the amount metabolized in the rat versus the mouse is smaller, probably due to saturation of metabolism in the rat. These inhalation results are comparable to those observed after oral administration of TCI.

8.3.2.1.3 Metabolic Profile. The experimental evidence is consistent with the metabolic pathways for TCI being qualitatively similar in mice, rats, and humans. In these species, the following principal end metabolites are common: trichloroacetic acid (TCA), trichloroethanol (TCE) and TCE-glucuronide, and their precursor metabolite, chloral. In recent studies, Green and Prout (1984) and Dekant et al. (1984) made comparative studies of biotransformation of TCI in rats and mice, and failed to detect any major species or strain differences in metabolism. In addition, TCI oxide has been demonstrated to be formed by both mouse and rat hepatic microsomes *in vitro* (Miller and Guengerich, 1982).

Dekant et al. (1984) isolated and identified, by rigorous methodology, the metabolites in exhaled air and urine of mice and rats given an oral dose of 200 mg/kg of ^{14}C-TCI. The metabolite profiles for these two species are shown in Table 4-11 (Chapter 4). Previously, TCE, its glucuronide, and TCA had been identified as urinary metabolites of TCI for mice, rats, and humans, and carbon dioxide had been identified as an end metabolite in the exhaled air of mice and rats (see Chapter 4). Hathaway (1980) detected traces of dichloroacetic acid in mouse urine, but only after very large (2 mL TCI/kg) oral doses. To explain these results, Hathaway proposed a "spillover" model, in which an excess of compound saturates "deactivation mechanisms" of the TCI oxide formed, producing an intermediate that becomes available for reactions that include spontaneous hydrolytic dechlorination to dichloroacetic acid (Kline and Van Duuren, 1977). However, Figure 4-8 shows that, at the dose of 200 mg TCI/kg, metabolism is far from saturated in mice (or rats). Dekant et al. (1984)

identified dichloroacetic acid in the urine of both mice and rats at this dose. In addition, these investigators identified two previously unrecognized metabolites, oxalic acid and N-(hydroxyacetyl)-aminoethanol (HAAE) in the urine of both of these species. They also identified significant amounts of HAAE in the urine of human volunteers exposed to 200 ppm TCI for 6 hours. There are no reported human studies in which carbon dioxide and oxalic acid, both normal endogenous compounds, have as yet been identified as specific metabolites of TCI, since doing so would require administration of labeled TCI. Nonetheless, the observations of Dekant et al., and of others, indicate that qualitatively, the pathways of TCI metabolism are similar in mice, rats, and humans (see Chapter 4 for a more detailed review).

Whether there is a quantitative difference in TCI metabolism in mice, rats, and humans is a question that remains unresolved. Dekant et al. (1984, Table 4-11) have noted that, in the case of the principal urinary metabolites, the ratio of TCE to TCA is much greater in the mouse than in the rat. However, they suggest that the smaller relative amounts of TCA in the urine of mice may be due to an active TCA enterohepatic recirculation, with TCA excreted in the feces in a conjugate form. In humans, the urinary ratio of TCE to TCA varies, but is generally found to be about 2:1.

8.3.2.1.4 Covalent Binding. Banerjee and Van Duuren (1978) compared covalent binding of ^{14}C-TCI to mouse and rat microsomes and observed that mice microsomes are two- to fourfold more active in metabolizing TCI to reactive covalent-binding metabolites than are rat microsomes. The covalent binding is proportional to the metabolism of TCI in these species. Stott et al. (1982) also investigated covalent binding *in vivo* after 6-hour exposures of mice and rats to 10 and 600 ppm ^{14}C-TCI. The time of maximum binding post-exposure was found to be 0 and 3 hours, respectively, for liver and kidney. These results are given in Table 8-32. At low exposure concentrations of TCI (10 ppm), rat and mouse covalent binding per μg of protein occurred to the same extent, with the amount of liver binding being two- to threefold greater than kidney binding. At high TCI exposures (600 ppm), which are metabolism-saturating concentrations for the rat (Tables 8-30 and 8-31), the amount of covalent binding was three- to fourfold greater per μg of protein in mice than in rats, but was in proportion to TCI metabolism; therefore, the ratios of total amounts of covalent binding in the rat versus the mouse approximates the relative metabolic rates in these species. Stott et al. also observed DNA aklylation in mice dosed with 1.2 g/kg high-specific ^{14}C-TCI. Their results showed that TCI or its metabolites are capable of binding to DNA, but that the binding ability was low when compared to dimethylnitrosamine and aflatoxin. Similar results have been reported by Parchman and Magee (1982), and Di Renzo et al. (1982).

8.3.2.2 Calculation of Human Equivalent Doses from Animal Data--The pharmacokinetic and metabolic data that most closely approximate the conditions of the NTP and NCI carcinogenicity bioassays are found in the following studies: 1) Prout et al. (1984), oral administration of single doses of TCI at dose levels up to 2,000 mg/kg to rats and mice; 2) Green and Prout (1984), chronic (180 days) oral administration of TCI at a dose level of 1,000 mg/kg/day to rats and mice; 3) Dekant et al. (1984), oral administration of a single dose of TCI at a dose level of 200 mg/kg to rats and mice; and 4) Buben and O'Flaherty (1985), chronic (6-week) oral administration of TCI at levels up to 2,000 mg/kg to mice. The comparative studies of TCI metabolism in both mice and rats of Prout et al. (1984), Green and Prout (1984), and Dekant et al. (1984), as well as

TABLE 8-32. COVALENT BINDING TO LIVER AND KIDNEY MACROMOLECULES OF ^{14}C-TCI IN MALE B6C3F1 MICE AND OSBORNE-MENDEL RATS

Exposure ppm	Tissue	Species	Binding pmole-eq per μg protein Av. of 4
10	liver	mouse	0.318
		rat	0.268
	kidney	mouse	0.168
		rat	0.153
600	liver	mouse	20.4
		rat	4.72
	kidney	mouse	5.6
		rat	1.77

SOURCE: Adapted from Stott et al., 1982.

the inhalation studies of Stott et al. (1982), provide evidence that, for a given dose, such as those used in the NCI and NTP bioassays, the magnitude of TCI metabolism in these species closely approximates their relative surface areas. For the risk calculation, it is assumed that the amount of TCI metabolized per m^2 surface area is equivalent among species.

The carcinogenicity data of the NTP and NCI bioassays show, in male and female B6C3F1 mice, a significant dose-related excess incidence of hepatocellular carcinomas at time-weighted average dosage levels of 1,000 mg/kg in the NTP study, and "low" and "high" doses for female and male mice in the NCI study of 869 and 1,739 mg/kg, and 1,169 and 2,339 mg/kg, respectively (Table 8-26). The data of Buben and O'Flaherty (1985, Figure 4-8) clearly indicate that, in Swiss-Cox mice, the metabolism of TCI is linearly proportional to oral doses up to at least 1,600 mg/kg. Hence, except for the "high" doses (1,730 and 2,339 mg/kg) given to female and male mice in the NCI study, the amount of metabolites formed and contributing to a carcinogenic response is directly proportional to the dose, which in turn, may be proportional to the tumorigenic response. Therefore, the dose-metabolism curve of Buben and O'Flaherty can be used in calculating TCI metabolism from the bioassay doses and in correcting for the saturation metabolism associated with the "high" doses of the NCI bioassay. However, since the Buben and O'Flaherty dose-metabolism curve was based solely on urinary metabolites (and hence underestimates the total amount of metabolism), a more definitive measure of metabolism, using ^{14}C-TCI as in the studies of Prout et al. (1984) and Green and Prout (1984) can be used to estimate the total TCI metabolism associated with the bioassay doses. Also, urinary metabolites are assumed to be linearly proportional to total metabolism.

8.3.2.2.1 NTP Study. In this study, the time-weighted average gavage dose was 1,000 mg/kg/day, or, based on the average terminal weight for the male and female mice, 40 mg/male mouse/day and 35 mg/female mouse/day (Table 8-26). In other studies, with similar doses given either singly or chronically in corn oil to B6C3F1 mice, virtually complete absorption occurred; a portion of the dose was excreted unchanged in exhaled air (Prout et al., 1984; Green and Prout, 1984; Dekant et al., 1984). On the basis of these data, the fractional dose metabolized for oral doses of between 30 to 60 mg per animal averages 78%. These doses are on the linear portion of the dose-metabolism curve for mice of Buben and O'Flaherty (1984, Figure 4-8). Hence, the daily body metabolic load (78% of the administered dose) for the mice in the NTP study is 780 mg/kg.

The relationship between the experimental administered dose (mg/animal) and the amount metabolized is estimated on the basis of data from Prout et al. (1984) (see Table 8-27), using the "Michaelis-Menten" type of equation, $M = a \times d/(b + d)$, where d is the experimental administered dose (mg/animal) and M is the corresponding amount metabolized. The parameters a and b are determined by least-square estimates and are equal to 594.1 mg and 702.97 mg, respectively, with a coefficient of determination $r^2 = 0.99$. This equation is used to convert a bioassay dose in mice to the metabolized dose. Assuming a lifetime of 104 weeks, the daily lifetime average effective exposure (LAE) of the mice is given by:

$$LAE_M = \frac{104 \text{ weeks}}{104 \text{ weeks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \text{metabolized dose, mg/animal/day}$$

and the equivalent dose for humans,

$$LAE_H = LAE_M \times \text{scaling factor}$$

The scaling factor for mouse to humans is: $(W_H/W_A)^{2/3}$, where W_H and W_A are, respectively, the human and animal body weights;

male mouse $(70/0.040)^{2/3} = 145.21$; female mouse $(70/0.035)^{2/3} = 158.74$

The lifetime average human equivalent doses for the NTP mouse carcinogenicity bioassay of TCI, expressed in units of mg/day, mg/m^2 surface area/day, and mg/kg/day, are presented in Table 8-33.

However, a total daily dose administered as a single bolus, such as in a bioassay, may not be biologically equivalent to the same total daily dose resulting from a continuous 24-hour exposure (e.g., air pollutant) or from several intermittent smaller doses (e.g., drinking water). As has been pointed out by Beliles (1975), a 24-hour continuous exposure to a given concentration of an atmospheric pollutant is not necessarily the simple equivalent of one-fourth the 8-hour workplace exposure.

8.3.2.2.2 NCI Study. For this study, the time-weighted average gavage doses ("high" and "low" doses) in mg/kg/day given to male and female B6C3F1 mice are given in Table 8-26, and the corresponding dose per single animal per day is given as calculated from the average terminal weight for the male and female mice of 33 g and 26 g, respectively. As noted previously, doses in corn oil to B6C3F1 mice in this range have been observed experimentally, when given as a single oral dose or chronically (daily for 180 days), to be completely absorbed from the GI tract, although a portion of the dose effects a first-pass of the liver and is excreted in exhaled air unchanged (Prout et al., 1984; Green and Prout, 1984; Dekant et al., 1984). The data of these investigators indicate that the percentage of TCI dose metabolized for oral doses of between 30 and 60 mg per animal averages 78% (Table 8-27). Furthermore, these doses are on the

TABLE 8-33. DOSE CONVERSION FOR THE NTP BIOASSAY (1982) IN B6C3F1 MICE

Sex	Oral experimental dose (time-weighted)		Animal metabolized dose	Human equivalent dose[a] (lifetime average exposure)			Tumor incidence
	mg/kg/day	mg/day	mg/day	mg/day	$mg/m^2/day$[b]	mg/kg/day	
Male	0	0	0	0	0	0	8/48
	1,000	40	31.98	3,317.23	1,793.10	47.39	30/50
Female	0	0	0	0	0	0	2/49
	1,000	35	28.17	3,194.06	1,726.52	45.62	13/49

[a] Assuming the animals were exposed to TCI for their entire lifetimes, human equivalent dose (mg/day) = animal metabolized dose (mg/day) x 5/7 x $(W_H/W_A)^{2/3}$

where W_A = 0.040 kg; terminal average weight for male mice
0.035 kg; terminal average weight for female mice

W_H = 70 kg; weight for standard man

The factor 5/7 reflects the fraction of days per week that the animals received treatment.

[b] Surface area for standard man = 1.85 m^2 (Diem and Lentner, 1970).

linear portion of the dose-metabolism curve for mice of Buben and O'Flaherty (1984). However, the higher doses of 1,739 and 2,339 are on the nonlinear, "saturating" portion of the curve and, as estimated from this curve (Figure 4-8), further adjustments of 96% and 68%, respectively, are appropriate, assuming the same curve for the B6C3F1 strain mouse. The daily amounts of dose metabolized for the mice in the NCI study are estimated below in the same manner as was done for the NTP study (see Table 8-34), using the data from the Prout et al. (1984) study, and by also including the data from Buben and O'Flaherty, which affects the higher dose only, for comparison. The data from the Buben and O'Flaherty study are especially important to consider because this study reflects a chronic dosing schedule, making it more relevant to the conditions of the NTP and NCI carcinogenicity studies. The daily lifetime average exposure (LAE) of the mice is given by

$$LAE_M = \frac{78 \text{ weeks}}{90 \text{ weeks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \text{amount metabolized, mg/animal/day}$$

and the equivalent dose for humans,

$$LAE_H = LAE_M \times \text{scaling factor}$$

The scaling factors for mouse to man $(W_H/W_A)^{2/3}$ are:

male mouse $(70/0.033)^{2/3} = 165.09$

female mouse $(70/0.026)^{2/3} = 193.53$

The lifetime average human equivalent doses for the NCI mouse carcinogenicity bioassay of TCI, expressed in units of mg/day, mg^2 surface area/day, and mg/kg/day, are presented in Table 8-34. The adjustment for partial lifetime in the NCI study, 90 weeks versus 104 weeks in the NTP study, is made later.

TABLE 8-34. DOSE CONVERSION FOR THE NCI BIOASSAY (1976) IN B6C3F1 MICE

Sex	Oral experimental dose (time-weighted)		Animal metabolized dose	Human equivalent dose[a] dose (lifetime average exposure)			Tumor incidence
	mg/kg/day	mg/day	mg/day	mg/day	mg/m^2/day[b]	mg/kg/day	
male	0	0	0	0	0	0	1/20
	1,169	38.58	30.90	3,157.93	1,706.98	45.11	26/50
	2,339	77.19	58.77 (39.96)[c]	6,006.21	3,246.60	85.80	31/48
female	0	0	0	0	0	0	0/20
	869	22.59	18.49	2,215.19	1,197.40	31.65	4/50
	1,739	45.21	35.89 (34.45)[c]	4,299.79	2,324.21	61.43	11/47

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[a] Human equivalent dose (mg/day) = animal metabolized dose (mg/day) x 5/7 x 78/90 x $(W_H/W_A)^{2/3}$

where W_A = 0.033 kg for male mice
= 0.026 kg for female mice

W_H = 70 kg; weight for standard man.

The factors 5/7 and 78/90 reflect the treatment schedule of 5 days per week for 78 weeks of a 90-week lifetime.

[b] Surface area for standard man = 1.85 m^2 (Diem and Lentner, 1970).

[c] Assuming urinary metabolites account for about 50% of total metabolism, the additional information provided by the Buben and O'Flaherty (1985) study would predict 40 mg/day (male) and 34.45 mg/day (females), a very small difference, as the amount metabolized at the high doses (this is comparable to the amounts predicted by fitting a curve to the Prout et al. [1984] data only, considering the differences in experimental conditions and mouse strains).

8.3.2.3 Summary--The parent chemical, TCI, is metabolized to electrophilic intermediate products that can react readily with cellular macromolecules, resulting in a spectrum of toxic responses that includes carcinogenesis. For example, the epoxide metabolite is considered sufficiently capable of producing the toxic end point of carcinogenicity.

Thus, the experimental data base for TCI provides some support for determining an extrapolation base for laboratory animals to man of $W^{2/3}$, and allows estimates of daily animal exposure (fractional dose metabolized) contributing to the tumorigenic response for the conditions of the NCI and NTP bioassays.

Estimates of equivalent human exposure can be calculated using the data from the experimental animal studies. The appropriate interspecies scaling is thus assumed to be on a surface area basis, i.e., $W^{2/3}$. It is of some interest also to view the estimates of equivalent human exposure that produced hepatocellular carcinomas in mice in perspective with human body metabolic loads from air exposure. Humans appear to metabolize inhaled TCI extensively, as reported by various investigators (see Chapter 4). The studies in human subjects indicate that at least 60% and possibly as much as 90% of the retained TCI from vapor exposures of less than 500 ppm (1,690 mg/m^3) is metabolized in the body. Thus, human subjects undergoing light physical activity, exposed for 8 hours to 150 ppm TCI in air, develop a body burden of about 2,500 mg (Monster et al., 1976, 1979; Nomiyama and Nomiyama, 1977; Fernandez et al., 1977). Nomiyama and Nomiyama (1977) observed that, at least for up to 300 ppm inhalation exposure, the metabolism of TCI is not saturated in humans. (See Chapter 4 for a detailed discussion of these studies).

8.3.3 Choice of Risk Model

8.3.3.1 General Considerations--The data used by the CAG for quantitative estimation are of two types: 1) lifetime animal studies, and 2) human studies in which excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses, with an incidence determined by the extrapolation model.

There is no solidly acceptable scientific basis for any mathematical extrapolation model that relates chemical carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time the dominant view of the carcinogenic process involves the concept that most agents that cause cancer also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a low-dose linearity and nonthreshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. At higher doses there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The low-dose linearity and nonthreshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, and liver cancer induced by aflatoxins in the diet). There is also some evidence from animal experiments that is consistent with the linear nonthreshold model (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large scale ED_{01} study at the National Center for Toxicological Research and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the nonthreshold model, which is linear at low doses, has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing a plausible upper limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the dose-risk relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-risk response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

The methods used by the CAG for quantitative assessment are consistently conservative, i.e., tending toward high estimates of risk. The most important part of the methodology contributing to this conservatism is the linear non-threshold extrapolation model. There are a variety of other extrapolation models that could be used, all of which would give lower risk estimates. These alternative models have not been used in the following analysis because, in the opinion of the CAG, the evidence of carcinogenicity for TCI does not warrant that complete an analysis. Furthermore, with the limited data available from these animal bioassays, especially at the high-dose levels required for testing, almost nothing is known about the true shape of the dose-response curve at low environmental levels. The position is taken by the CAG that the risk estimates obtained by use of the low-dose linear nonthreshold model are plausible upper limits, and that the true risk could be lower.

In terms of the choice of animal bioassay as the basis for extrapolation, where more than one acceptable study is available, a general approach is to use the most sensitive responder, i.e., the data set that gives the highest estimate of lifetime cancer risk, on the assumption that humans are as sensitive as the most sensitive animal species tested. For TCI, four sets of bioassay data in B6C3F1 mice, which produce comparable risk estimates, are used.

Extrapolation from animals to humans can be done on the basis of relative body weights, surface areas, metabolic rates, or other measures. The general approach is to use the extrapolation base (mg/kg, surface area, etc.) which can be appropriately justified by the available experimental data from animals and humans. However, it is usually not clear which extrapolation base is the most appropriate for the carcinogenic response per se. When there is insufficient or no experimental data to determine an extrapolation base either directly or indirectly, the most generally employed and conservative method is used, i.e., extrapolation from animal dose to a human equivalent dose on the basis of relative surface areas ($W^{2/3}$). For the TCI gavage studies in mice, the use of an extrapolation based on surface area ($W^{2/3}$) rather than body weight ($W^{1.0}$) would increase the unit risk estimates by a factor of 12 to 13. However, for TCI, experimental data on metabolism and kinetics are used in dose extrapolation from mouse to man.

8.3.3.2 Mathematical Description of the Low-Dose Extrapolation Model--Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[-(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d or the effect of treatment.

The point estimate of the coefficents q_i, $i = 0, 1, 2, \ldots, k$, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL83, developed by Howe (1983). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk, and R/q_1^* is a 95% lower confidence limit on the dose, producing an extra risk of R. Let

L_0 be the maximum value of the log-likelihood function. The upper limit, q_1^*, is then calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value, q_1^*, for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\,(L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear nonthreshold concept discussed earlier. The slope, q_1^*, is taken as an upper-bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.)

In fitting the dose-risk response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted, and the model is refitted to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i\,(1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

8.3.3.3 Adjustment for Less Than Lifespan Duration of Experiment--If the duraation of experiment L_e is less than the natural lifespan of the test animal L, the slope q_1^*, or more generally the exponent g(d), is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose d, is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the 2nd power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, we would expect the cumulative tumor rate to increase by at least the 3rd power of age. Using this fact, we assume that the slope q_1^*, or more generally the exponent g(d), would also increase by at least the 3rd power of age. As a result, if the slope q_1^* [or g(d)] is calculated at age L_e, we would expect that if the experiment had been continued for the full lifespan L, at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Daffer et al. (1980) where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp [-f(t) \times g(d)]$$

8.3.4 Calculation of Unit Risk

8.3.4.1 Definition of Unit Risk--This section deals with the unit risk for TCI in air and water and the potency of TCI relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air or water pollutant is defined as the increased lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 $\mu g/m^3$ of the agent in the air they breathe, or to 1 $\mu g/L$ in the water they drink. This calculation is done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk which might be associated with air or water exposure to these agents, if the actual exposures are known.

8.3.4.2 Slope Calculation--Using the incidence data in Tables 8-33 and 8-34 and the corresponding human equivalent metabolized doses, the slope estimate, q_1^*, for TCI is calculated using the linearized multistage model, as shown in Table 8-35. The q_1^* value is used to compare relative potencies of carcinogens and to calculate the unit risks for drinking water and air.

Since NCI mice were followed for only 90 weeks, rather than for 104 weeks as in the NTP study, the risk estimates calculated on the basis of NCI mice are multiplied by a factor $(104/90)^3 = 1.54$. The rationale for such an adjustment is given in Section 8.3.3.3. The slope estimates in the last column of Table 8-35 are expressed in terms of the ambient concentration. These values are derived by using the calculated metabolized dose-response curve and the dose-metabolism relationship previously established. Since both the metabolized dose-response and dose-metabolism curves are linear at low doses, the resultant

TABLE 8-35. SLOPE ESTIMATES (q_1^*)[a] BASED ON EXTRAPOLATION FROM DATA IN MALE AND FEMALE MICE

Data base	q_1^* (animal)[b] (mg metabolized dose/kg/day)$^{-1}$	q_1^* (human) (mg metabolized dose/kg/day)$^{-1}$	q_1^* (human) (mg administered dose/kg/day)$^{-1}$
NTP:			
Male mice	1.8×10^{-3}	2.2×10^{-2}	1.9×10^{-2}
Female mice	7.5×10^{-4}	9.5×10^{-3}	8.0×10^{-3}
NCI:			
Male mice	1.6×10^{-3}	2.1×10^{-2}	1.8×10^{-2}
Female mice	5.0×10^{-4}	6.9×10^{-3}	5.8×10^{-3}
Geometric mean	1.0×10^{-3}	1.3×10^{-2}	1.1×10^{-2}

[a] q_1^* is defined as the 95% upper limit of the linear component (slope) in the multistage model. Since the dose-response curve is virtually linear below 1 mg/kg/day, the slope is numerically equal to the upper limit of the incremental lifetime risk estimates at 1 mg/kg/day. The slope values are used to compare relative carcinogenic potencies and to calculate the unit risks for drinking water and air.

[b] q_1^* (animal) is calculated using the daily lifetime average effective exposure (LAE_M).

dose-response curve is also linear at low ambient doses. Because the slope estimates from all four data sets are comparable, the geometric mean of these four numbers is used to represent the slope, q_1^*, for TCI. The q_1^* values calculated from the NCI and NTP studies are presented in the last two columns of Table 8-35, expressed in terms of metabolized dose and also in terms of ambient dose after adjusting for metabolism. The geometric mean is $q_1^* = 1.3 \times 10^{-2}/(\text{mg/kg/day})$ in terms of the metabolized dose, or $q_1^* = 1.1 \times 10^{-2}/(\text{mg/kg/day})$ in terms of the ambient dose. These q_1^* values are used to calculate the unit risks for water and air.

8.3.4.3 Risk Associated with 1 μg/L of TCI in Drinking Water--The daily dose (mg/kg/day) from consumption of 2 L of water containing 1 μg/L of TCI is calculated as follows:

$$\begin{aligned} d &= (1\ \mu\text{g/L}) \times (2\ \text{L/day}) \times (10^{-3}\ \text{mg}/\mu\text{g})/70\ \text{kg} \\ &= 2.9 \times 10^{-5}\ \text{mg/kg/day} \end{aligned}$$

Here it is assumed that, on the average, humans weigh 70 kg and consume 2 L of water each day. Therefore, the incremental lifetime risk associated with 1 μg/L of TCI in drinking water is:

$$\begin{aligned} p &= 1.1 \times 10^{-2} \times 2.9 \times 10^{-5} \\ &= 3.2 \times 10^{-7} \end{aligned}$$

where 1.1×10^{-2} is the human carcinogenic potency in terms of adjusted ambient dose, taken from Table 8-35.

8.3.4.4 Risk Associated with 1 μg/m³ of TCI in Air--Two animal inhalation studies showed positive results in mice. These were the Henschler et al. (1980) study, showing increased malignant lymphomas in females, and the Bell et al. (1978) study showing increased hepatocellular carcinomas in males. As discussed in the qualitative section, both studies exhibited serious defects. The Bell et al. (1978) study, which showed increased hepatocellular carcinomas in male mice is used to calculate the unit risk for air. Because of the deficiencies of this study, the risk estimate is used only to compare with those calculated on the basis of gavage studies. In order to use the potency q_1^* from the gavage studies, it is necessary to estimate the amount metabolized when a subject is exposed to 1 $\mu g/m^3$ of TCI in air.

8.3.4.4.1 Unit Risk Estimate Calculated on the Basis of Human Metabolized Dose. Although there are many studies on metabolism of TCI in humans, most of the studies did not present the relationship between TCI uptake and the amount metabolized. The study by Monster et al. (1976) is one which can be used for estimating the amount metabolized when a subject is exposed to 1 $\mu g/m^3$ of TCI in air. The study presents the total uptake and the percentage excreted as TCI in the exhaled air for four subjects who were exposed to 70 ppm and 140 ppm for 4 hours, including two half-hour 100-watt exercises. Since results from 70 ppm and 140 ppm are comparable, the results for the 70 ppm group are used for risk calculation. These results are as follows:

Subject	Uptake (mg)	Percentage of dose exhaled as TCI	Total dose metabolized (mg)
A	500	9	455.0
B	450	17	373.5
C	470	10	423.0
D	660	8	607.2

The median amount metabolized for the four subjects is 439 mg. Under the assumption that the dose metabolized is linearly related to the intensity and duration of exposure, the dose metabolized corresponding to 1 $\mu g/m^3$ of TCI in air is

$$d = 439 \text{ mg} \times (24 \text{ hours}/4 \text{ hours}) / (70 \text{ ppm} \times 5{,}475\ \mu g/m^3/\text{ppm})$$
$$= 6.9 \times 10^{-3} \text{ mg}$$

where 1 ppm = 5,475 $\mu g/m^3$. That is, under a continuous (24 hours) exposure to 1 $\mu g/m^3$ of TCI in air, the body metabolic load is estimated to be

$$d = 6.9 \times 10^{-3}/70 = 9.9 \times 10^{-5} \text{ mg/kg/day}$$

Therefore, the unit risk for TCI in air is

$$P = 1.3 \times 10^{-2} \times 9.9 \times 10^{-5}$$
$$= 1.3 \times 10^{-6}$$

where 1.3×10^{-2} is the human carcinogenic potency in terms of metabolized dose, taken from Table 8-35. This unit risk estimate is comparable to the other three estimates to be calculated by different approaches and/or on different data bases. The CAG recommends that $P = 1.3 \times 10^{-6}$ be used as the unit risk estimate for TCI in air because it is calculated on the basis of human metabolized dose.

8.3.4.4.2 Unit Risk Estimate Calculated on the Basis of Animal Metabolized Dose. Stott et al. (1982) exposed rats and mice to 10 ppm and 100 ppm of ^{14}C-TCI for 6 hours and estimated the amount metabolized by collecting the radioactivity excreted in urine, feces, expired air, etc., and by determining TCI in the expired air for 50 hours post-exposure. The results of 10 ppm exposure, which is more relevant to the risk calculation, are reproduced below from Tables 8-30 and 8-31.

	Mice	Rats
TCI expired (mg)	0.003	0.03
Metabolized (mg)	0.36 (99%)	1.15 (98%)
Total uptake (mg)	0.36	1.18

These data can be used in two different ways to estimate human risk due to 1 $\mu g/m^3$ of TCI in air.

Approach 1: Since the body metabolic loads for mice after oral and inhalation exposure are known, it is possible to estimate the unit risk by inhalation using the slope q_1^* (animal) = $1.0 \times 10^{-3}/(mg/kg/day)$, calculated on the basis of a gavage study (Table 8-35). For mice, 10 ppm in air for 6 hours resulted in a metabolized dose of 0.36 mg. Under the assumption that the amount metabolized at low doses is linearly related to the intensity and duration of exposure, the daily dose metabolized corresponding to 1 $\mu g/m^3$ continuous exposure of TCI in air is:

$$0.36 \text{ mg} \times (24 \text{ hours}/6 \text{ hours}) / (10 \text{ ppm} \times 5{,}475\ \mu g/m^3/\text{ppm})$$
$$= 2.63 \times 10^{-5} \text{ mg}$$

where 1 ppm = 5,475 $\mu g/m^3$. That is, if a mouse is exposed continuously to 1 $\mu g/m^3$ of TCI in air, its body metabolic load is 2.63×10^{-5} mg/day or 7.5×10^{-4} mg/kg/day for a mouse weighing 0.035 kg. Therefore, the lifetime incremental risk for mice due to exposure to 1 $\mu g/m^3$ of TCI in air is

$$P \text{ (mice)} = 1.0 \times 10^{-3} \times 7.5 \times 10^{-4}$$
$$= 7.5 \times 10^{-7}$$

To convert the risk estimate from mice to humans, the human concentration $C(\mu g/m^3)$ that is equivalent to mouse exposure to 1 $\mu g/m^3$ of TCI must be determined. Under the assumption that the amount metabolized per body surface area is equivalent between mice and humans, the human concentration $C(\mu g/m^3)$ must satisfy the relationship

$$\frac{C \times 20\ m^3/day}{(70\ kg)^{2/3}} = \frac{1 \times 0.043\ m^3/day}{(0.035\ kg)^{2/3}}$$

where 20 m^3/day and 0.043 m^3/day are assumed to be, respectively, the volumetric breathing rates for a human weighing 70 kg and a mouse weighing 0.035 kg. As a consequence, $C = 0.34\ \mu g/m^3$. That is, the exposure concentration, 0.34 $\mu g/m^3$, for humans is considered equivalent to that for mice at 1 μ/m^3. Therefore, the unit risk estimate for humans is

$$P = 7.5 \times 10^{-7}/0.34$$
$$= 2.2 \times 10^{-6}$$

This value is comparable to that calculated on the basis of actual human data.

Approach 2: In Approach 1, only mouse data are used. As an alternative approach, the relationship of the metabolized doses between mice and rats could be used to infer the human metabolized dose. As demonstrated previously, the relative amount of dose metabolized between mice and rats is approximately equal to two-thirds of the ratio of body weights. Assuming that the same relationship holds for humans and mice, the human metabolized dose corresponding to 1 $\mu g/m^3$ of TCI in air would be

$$d = 2.63 \times 10^{-5}\ mg/day \times (70\ kg/0.035\ kg)^{2/3}$$
$$= 4.17 \times 10^{-3}\ mg/day \text{ or equivalently}$$
$$d = 4.17 \times 10^{-3}/70 = 5.96 \times 10^{-5}\ mg/kg/day$$

where 2.63×10^{-5} mg/day is the metabolized dose for mice corresponding to 1 μg/m^3 of TCI in air, as calculated previously in Approach 1. Therefore, the unit risk is estimated to be

$$P = 1.30 \times 10^{-2} \times 5.96 \times 10^{-5}$$
$$= 7.7 \times 10^{-7}$$

where $q_1^* = 1.30 \times 10^{-2}/(\text{mg/kg/day})$ is the slope for humans taken from Table 8-35. This risk estimate is again comparable to those calculated previously.

8.3.4.4.3 Unit Risk Estimate Calculated on the Basis of an Inhalation Study in Mice. The Bell et al. (1978) study, which showed increased hepatocellular carcinomas in male mice, can be used to calculate the unit risk for TCI in air. Because of the deficiencies in this study, the risk calculated from this study should be considered crude and is to be used only for comparision with those calculated on the basis of gavage studies. For both gavage and inhalation studies, the animal species used in the experiment and the observed tumor site and type are identical. Animals in the Bell et al. (1978) study were exposed to TCI 6 hours/day, 5 days/week, for 24 months. The dose and response data are as follows:

Experimental dose (ppm)	0	100	300	600
LAD (ppm)[a]	0	17.86	53.57	107.14
Hepatocellular carcinoma incidence	18/99	28/95	31/100	43/97

[a] The lifetime average dose (LAD) is calculated by LAD = dose (ppm) x (5 days/7 days) x 6 hours/24 hours.

Using the linearized multistage model, the carcinogenic potency (the 95% upper limit of the linear coefficient in the multistage model) for animals is calculated to be

$$q_1^* \text{ (animal)} = 4.8 \times 10^{-3}/\text{ppm}$$

or

$$q_1^* \text{ (animal)} = 4.8 \times 10^{-3}/5475$$
$$= 8.8 \times 10^{-7}/(\mu g/m^3)$$

using the fact that 1 ppm = 5475 $\mu g/m^3$.

To convert the potency from animals to humans, it is assumed that dose relative to body surface area is equivalent among species. As calculated previously (see Section 8.3.4.4.2), the exposure concentration C ($\mu g/m^3$) for humans that is equivalent to 1 $\mu g/m^3$ for mice is C = 0.34 $\mu g/m^3$. Therefore, the carcinogenic potency for humans is

$$q_1^* = 8.8 \times 10^{-7}/0.34$$
$$= 2.6 \times 10^{-6}/(\mu g/m^3)$$

The risk due to 1 $\mu g/m^3$ of TCI in air is thus calculated to be

$$P = 2.6 \times 10^{-6}/(\mu g/m^3) \times 1 \ \mu g/m^3$$
$$= 2.6 \times 10^{-6}$$

This risk estimate is also comparable to those calculated by means of different approaches and on the basis of different data bases.

8.3.4.5 Discussion--To the extent possible, the available metabolism and kinetic information for TCI has been used in the risk calculations. The use of this information may or may not reduce the uncertainties that are associated with the various steps involved in risk calculations. There are three major aspects of uncertainty in the risk assessment of TCI:

1. Extrapolation from high dose to low dose (i.e., low-dose extrapolation)
2. Extrapolation from animals to humans (i.e., species conversion), and
3. Extrapolation from gavage to inhalation (i.e., route-to-route extrapolation.

In calculating the dose-response relationship for TCI, the amount metabolized is considered to be an effective dose. The use of this surrogate effective dose may not eliminate the uncertainty associated with the low-dose extrapolation because the dose actually reaching the target sites may not be linearly proportional to the total amount metabolized, and the shape of the dose-response relationship is still unknown. However, it seems reasonable to expect that the uncertainty with regard to the low-dose extrapolation would be somewhat reduced by the use of the metabolized dose because the metabolized dose better reflects the dose-response relationship, particularly within the high-dose region.

To extrapolate from animals to humans, the amount metabolized relative to body surface area is assumed to be equivalent (i.e., equally potent) among species. This assumption is by no means supported by the empirical data. For TCI, there is some evidence showing that, for a given dose in mg/kg (by the oral route) or ppm (by the inhalation route), the amounts metabolized relative to body surface area are approximately equal among species. However, there are no observations which support the proposition that the metabolized dose relative to body surface area is equally effective in inducing tumors among different species. An alternative approach would be to assume that mg metabolized dose/kg/day is equivalent among species. If this assumption is made, the slope factor q_1^*, as calculated for TCI and expressed in terms of $(\text{mg/kg/day})^{-1}$, would be reduced by approximately 10 times.

The slope calculated from the gavage study, using the metabolized dose, provides a better basis for extrapolating a risk estimate from the gavage to the inhalation route. This is so because the relationship between the ambient air concentration and the amount metabolized for TCI is available for both humans and animals. Therefore, the uncertainty due to route-to-route extrapolation should be reduced by the use of metabolized dose.

8.3.4.6 Interpretation of Unit Risk Estimates--For several reasons, the upper-limit unit risk estimate based on animal bioassays is only an approximate indication of the real risk in populations exposed to known carcinogen concentrations. First, there may be important differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. In addition, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative carcinogenic potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, although ordinarily the risk should be independent of route of exposure except in special circumstances, for example, nasal or lung carcinomas with inhalation exposure, or forestomach tumors with gavage administration.

The quantitative aspect of carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper-limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates for TCI presented in this chapter should not be regarded as immutable representations of the true cancer risks; however, the estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful. Table 8-35 gives the upper-limit slope estimates, q_1^*, from the linearized multistage model. The slope estimate can be used to compare the relative carcinogenic potency of TCI to that of other potential human carcinogens. The slope is also used to calculate upper-bound incremental risks at low levels of exposure.

8.4 RISK ESTIMATION FROM EPIDEMIOLOGIC DATA

8.4.1 Selection of Epidemiologic Data Sets

Whenever possible, human data are used in preference to animal bioassay data. If epidemiologic studies and sufficiently valid exposure information are available for a compound, they may be used in several ways. If these data show a carcinogenic effect, they are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose. If no carcinogenic effects are seen in the epidemiologic studies when positive animal evidence is available, the assumption is made that a risk does exist, but that it is too small to be observable in the epidemiologic data. An upper limit to the cancer incidence is then calculated, assuming hypothetically that the true incidence is below the level of detection in a human cohort of the size studied.

Very little information exists that can be used for extrapolating from high-exposure occupational studies to low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures. Thus, the Axelson et al. (1978) study provides data that may be used to calculate 95% upper-limit estimates on relative risk, although this study does not provide evidence for the carcinogenicity of TCI. Calculations may be based on the subcohort data from the Axelson et al. study, with particular focus on the low-exposure group, which had a latency period of at least 10 years.

8.4.2 Description of Risk Model

In human studies, the response is measured in terms of the relative risk of the exposed cohort as compared to the control group. The mathematical model employed assumes that for low exposures the lifetime probability of death from a specific cancer, P_0, may be represented by the linear equation

$$P_0 = A + B_H x$$

where A is the lifetime probability in the absence of the agent, and x is the average lifetime exposure to environmental levels in some units, say ppm. The factor, B_H, is the increased probability of cancer associated with each unit increase of the agent in air.

If we make the assumption that R, the relative risk of lung cancer for exposed workers compared to the general population, is independent of the length or age of exposure but depends only upon the average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B_H (x_1 + x_2)}{A + B_H x_1}$$

or

$$RP_0 = A + B_H (x_1 + x_2)$$

where x_1 = lifetime average daily exposure to the agent for the general population, x_2 = lifetime average daily exposure to the agent in the occupational setting, and P_0 = lifetime probability of dying of cancer with no or negligible TCI exposure.

Substituting $P_0 = A + B_H x_1$ and rearranging gives

$$B_H = P_0 (R - 1)/x_2$$

To use this model, estimates of R and x_2 must be obtained from the epidemiologic studies. The value P_0 is derived from the age-cause-specific death rates for combined males found in the U.S. Vital Statistics tables using the life table methodology.

8.4.3 Calculation of Upper Limits of Risk

Although the epidemiologic studies on TCI provide no evidence for its carcinogenicity, they can still be used to provide information on an upper limit of risk to be expected. The CAG adopts this procedure of using negative epidemiologic studies for providing upper limits when animal studies are positive. Such use of negative epidemiologic studies can modify estimates of potency from the positive animal studies.

Table 8-36 shows 95% upper-limit estimates on relative risk, as derived from data in Table 8-24 obtained by Axelson et al. (1978). These upper-limit estimates are calculated according to the following approach:

TABLE 8-36. NUMBER OF WORKERS WITH CANCER DEATHS

Cohort	Observed X	Upper limit u	Expected if no effect	Relative risk 95% upper limit
Total cohort	11	19.7	14.5	1.4
Subcohort with 10-year latency (high and low exposure)	9	17.2	9.5	1.8
High exposure	3	8.7	1.8	4.8
Low exposure	6	13.3	7.7	1.7

The 95% upper limit = u/expected number of cancer deaths, where u is the solution to the sum of Poisson probabilities, as follows:

$$\sum_{i=0}^{X} \frac{e^{-u}\,(u)^{i}}{i!} = 0.025$$

One might also interpret u as the upper limit of the number of workers with cancer deaths given the observed frequency, and X = number of observed workers with cancer deaths.

These upper-limit relative risks can be used to provide estimates of the the upper-limit probability of death from cancer due to a lifetime exposure to 1 $\mu g/m^3$ of TCI. This upper-limit probability estimate is 1.7×10^{-5} per $\mu g/m^3$ of TCI in air, and it is approximately 10 times greater than the risk estimate based on the NCI (1976) and NTP (1982) carcinogenicity gavage bioassays. In showing these risk calculations below, the CAG emphasizes that:

1. This upper-limit value is derived from a negative epidemiologic study. Not only was there no specific target organ for cancer, but the total number of cancer deaths was less than expected.
2. Since there was no specific target organ, the upper-limit lifetime probability estimate is based on all cancer deaths. Since the lifetime probability of death from cancer to humans is 0.19 (based on 1977 U.S. death rates, race and sex combined), any even moderately conservative confidence limit will provide a large upper-limit risk.

The calculations are based on the subcohort data from the Axelson et al. (1978) study, specifically the low exposure group with at least a 10-year latency period. Table 8-36 projects an upper-limit relative risk of 1.7. Since "low exposure" referred to exposures not exceeding concentrations of 100 mg TCA per mL in urine on the average, which, according to Axelson et al. (1978) corresponds to an ambient air exposure of no more than 30 ppm TCI in air, 30 ppm is used as the 8-hour time-weighted average dose. This corresponds to 30 ppm x 5,475 ($\mu g/m^3$)/ppm = $1.64 \times 10^5\ \mu g/m^3$. A lower dose estimate would provide an even higher upper-limit probability of death.

In the category of years exposed, in the absence of any other information, 10 years was chosen as a point assumed to be somewhere in the middle of the range.

On the basis of the above information, the total lifetime dose of TCI to the low-dose exposure workers is estimated as:

$$\frac{(0\text{ ppm} + 1.64 \times 10^5)}{2} \times \frac{240\text{ days}}{365\text{ days}} \times \frac{10\text{ years}}{70\text{ years}} = 7.7 \times 10^3\ \mu g/m^3$$

The $1.64 \times 10^5\ \mu g/m^3$ TCI of the working day is averaged with the $0\ \mu g/m^3$ of the non-working day, assuming that half of the air breathed during a day will be during working hours. The value 7.7×10^{-3} $\mu g/m^3$ is assumed to be a continuous exposure concentration. Thus, the upper-limit of the probability of death from 1 $\mu g/m^3$ TCI in air (continuous exposure) is estimated as:

$$P = \frac{P_0\ (R - 1)d_1}{d_2} = \frac{0.19\ (1.7 - 1)1}{7.7 \times 10^3} = 1.7 \times 10^{-5} \text{ per } \mu g/m^3$$

where R = the relative risk, d_1 = 1 $\mu g/m^3$, d_2 = dose of workers, and P_0 = the background lifetime probability of death from all cancers.

Based on a comparison of this upper-limit estimate with that from the animal studies, the Axelson et al. study is seen to provide no evidence that the carcinogenicity potency of TCI is less for humans than for animals.

8.5 RELATIVE CARCINOGENIC POTENCY

8.5.1 Derivation

One of the uses of the concept of unit risk is to compare the relative potencies of carcinogens. To estimate relative potency on a per mole basis, the unit risk slope factor is multiplied by the molecular weight, and the resulting number is expressed in terms of $(\text{mmol/kg/day})^{-1}$. This is called the relative potency index.

Figure 8-15 is a histogram representing the frequency distribution of potency indices of 54 chemicals evaluated by the CAG as suspect carcinogens. The actual data summarized by the histogram are presented in Table 8-37. Where human data are available for a compound, they have been used to calculate the index. Where no human data are available, animal oral studies and animal inhalation studies have been used in that order. Animal oral studies are selected over animal inhalation studies because most of the chemicals have animal oral studies; this allows potency comparisons by route.

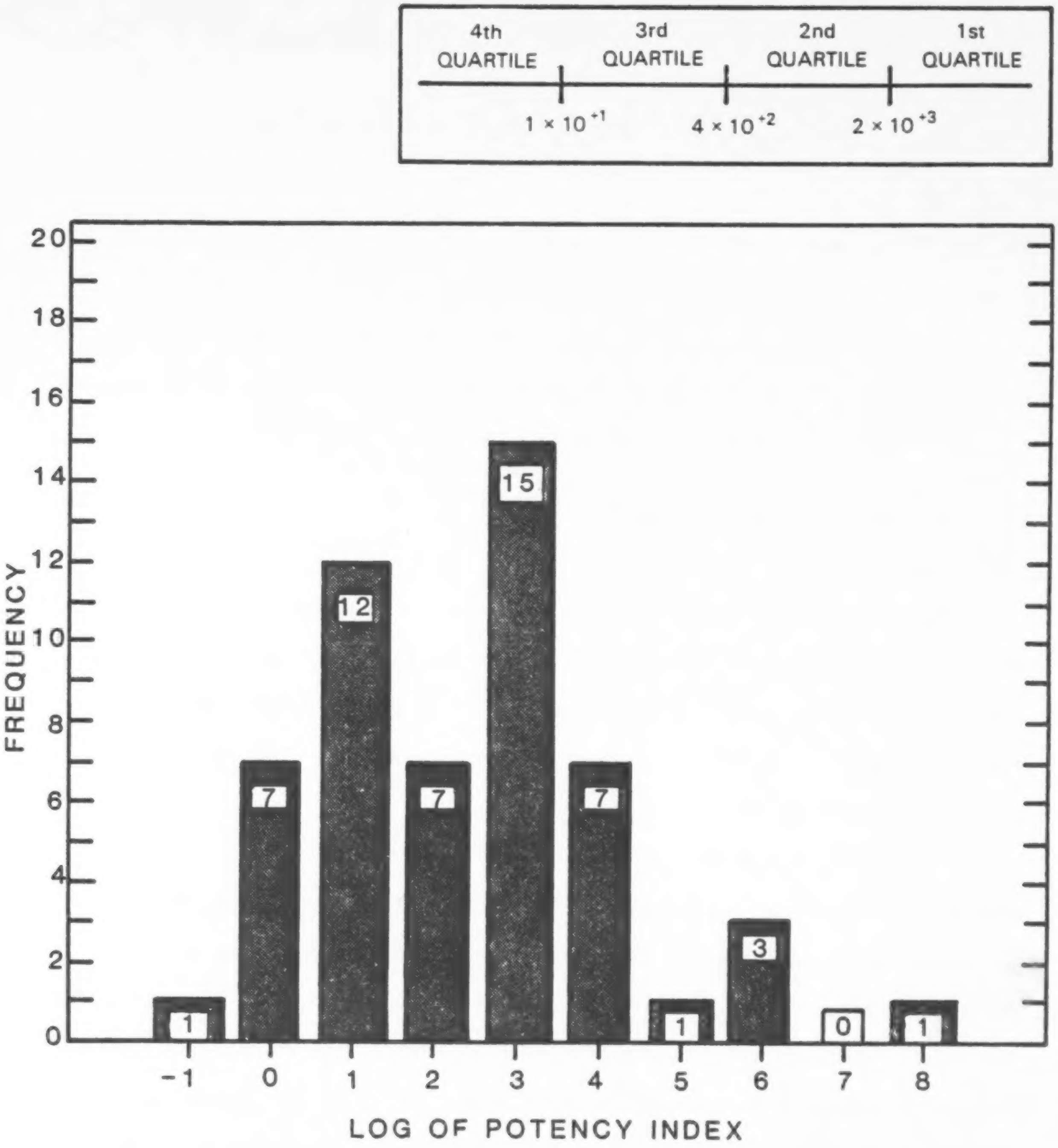

Figure 8-15. Histogram representing frequency distribution of the potency indices of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 8-37. RELATIVE CARCINOGENIC POTENCIES AMONG 54 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
		Humans	Animals					
Acrylonitrile	107-13-1	L	S	2A	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	1162-65-8	L	S	2A	2900	312.3	$9x10^{+5}$	+6
Aldrin	309-00-2	I	L	2B	11.4	369.4	$4x10^{+3}$	+4
Allyl chloride	107-05-1				$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	7440-38-2	S	I	1	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	50-32-8	I	S	2B	11.5	252.3	$3x10^{+3}$	+3
Benzene	71-43-2	S	S	1	$2.9x10^{-2}$(W)	78	$2x10^{0}$	0
Benzidene	92-87-5	S	S	1	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	7440-41-7	L	S	2A	2.6	9	$2x10^{+1}$	+1
1,3-Butadiene	106-99-0	I	S	2B	$1.0x10^{-1}$(I)	54.1	$5x10^{0}$	+1
Cadmium	7440-43-9	L	S	2A	6.1(W)	112.4	$7x10^{+2}$	+3
Carbon tetrachloride	56-23-5	I	S	2B	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	57-74-9	I	L	3	1.61	409.8	$7x10^{+2}$	+3

(continued on the following page)

TABLE 8-37. (continued)

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope[b] (mg/kg/day)-1	Molecular weight	Potency index[c]	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
Chlorinated ethanes								
1,2-Dichloroethane	107-06-2	I	S	2B	9.1×10^{-2}	98.9	9×10^{0}	+1
hexachloroethane	67-72-1	I	L	3	1.42×10^{-2}	236.7	3×10^{0}	0
1,1,2,2-Tetrachloroethane	79-34-5	I	L	3	0.20	167.9	$3\times10^{+1}$	+1
1,1,2-Trichloroethane	79-00-5	I	L	3	5.73×10^{-2}	133.4	8×10^{0}	+1
Chloroform	67-66-3	I	S	2B	7×10^{-2}	119.4	8×10^{0}	+1
Chromium VI	7440-47-3	S	S	1	41(W)	100	$4\times10^{+3}$	+4
DDT	50-29-3	I	S	2B	0.34	354.5	$1\times10^{+2}$	+2
Dichlorobenzidine	91-94-1	I	S	2B	1.69	253.1	$4\times10^{+2}$	+3
1,1-Dichloroethylene (Vinylidene chloride)	75-35-4	I	L	3	1.16(I)	97	$1\times10^{+2}$	+2
Dichloromethane (Methylene chloride)	75-09-2	I	L	3	6.3×10^{-4}(I)	84.9	5×10^{-2}	-1
Dieldrin	60-57-1	I	S	2B	30.4	380.9	$1\times10^{+4}$	+4
2,4-Dinitrotoluene	121-14-2	I	S	2B	0.31	182	$6\times10^{+1}$	+2
Diphenylhydrazine	122-66-7	I	S	2B	0.77	180	$1\times10^{+2}$	+2
Epichlorohydrin	106-89-8	I	S	2B	9.9×10^{-3}	92.5	9×10^{-1}	0
Bis(2-chloroethyl)ether	111-44-4	I	S	2B	1.14	143	$2\times10^{+2}$	+2

(continued on the following page)

TABLE 8-37. (continued)

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope[b] (mg/kg/day)-1	Molecular weight	Potency index[c]	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
Bis(chloromethyl)ether	542-88-1	S	S	1	9300(I)	115	$1\times10^{+6}$	+6
Ethylene dibromide (EDB)	106-93-4	I	S	2B	41	187.9	$8\times10^{+3}$	+4
Ethylene oxide	75-21-8	L	S	2A	3.5×10^{-1}(I)	44.1	$2\times10^{+1}$	+1
Heptachlor	76-44-8	I	S	2B	3.37	373.3	$1\times10^{+3}$	+3
Hexachlorobenzene	118-74-1	I	S	2B	1.67	284.4	$5\times10^{+2}$	+3
Hexachlorobutadiene	87-68-3	I	L	3	7.75×10^{-2}	261	$2\times10^{+1}$	+1
Hexachlorocyclohexane								
technical grade					4.75	290.9	$1\times10^{+3}$	+3
alpha isomer	319-84-6	I	S	2B	11.12	290.9	$3\times10^{+3}$	+3
beta isomer	319-85-7	I	L	3	1.84	290.9	$5\times10^{+2}$	+3
gamma isomer	58-89-9	I	L	2B	1.33	290.9	$4\times10^{+2}$	+3
Hexachlorodibenzodioxin	34465-46-8	I	S	2B	$6.2\times10^{+3}$	391	$2\times10^{+6}$	+6
Nickel	7440-02-0	L	S	2A	1.15(W)	58.7	$7\times10^{+1}$	+2
Nitrosamines								
Dimethylnitrosamine	62-75-9	I	S	2B	25.9(not by q_1^*)	74.1	$2\times10^{+3}$	+3
Diethylnitrosamine	55-18-5	I	S	2B	43.5(not by q_1^*)	102.1	$4\times10^{+3}$	+4
Dibutylnitrosamine	924-16-3	I	S	2B	5.43	158.2	$9\times10^{+2}$	+3
N-nitrosopyrrolidine	930-55-2	I	S	2B	2.13	100.2	$2\times10^{+2}$	+2
N-nitroso-N-ethylurea	759-73-9	I	S	2B	32.9	117.1	$4\times10^{+3}$	+4

(continued on the following page)

TABLE 8-37. (continued)

Compounds	CAS Number	Level of evidence[a]		Grouping based on IARC criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude ($\log_{10}$ index)
		Humans	Animals					
N-nitroso-N-methylurea	684-93-5	I	S	2B	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	86-30-6	I	S	2B	$4.92x10^{-3}$	198	$1x10^{0}$	0
PCBs	1336-36-3	I	S	2B	4.34	324	$1x10^{+3}$	+3
Phenols								
2,4,6-Trichlorophenol	88-06-2	I	S	2B	$1.99x10^{-2}$	197.4	$4x10^{0}$	+1
Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	I	S	2B	$1.56x10^{+5}$	322	$5x10^{+7}$	+8
Tetrachloroethylene	127-18-4	I	L	3	$5.1x10^{-2}$	165.8	$8x10^{0}$	+1
Toxaphene	8001-35-2	I	S	2B	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	79-01-6	I	L/S	3/2B	$1.1x10^{-2}$	131.4	$1x10^{0}$	0
Vinyl chloride	75-01-4	S	S	1	$1.75x10^{-2}$(I)	62.5	$1x10^{0}$	0

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[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

[b] Animal slopes are 95% upper-bound slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available. The slope value is an upper bound in the sense that the true value (which is unknown) is not likely to exceed the upper bound and may be much lower, with a lower bound approaching zero. Thus, the use of the slope estimate in risk evaluations requires an appreciation for the implication of the upper bound concept as well as the "weight of evidence" for the likelihood that the substance is a human carcinogen.

[c] The potency index is a rounded-off slope in $(mmol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

8.5.2 Potency Index

The potency index for TCI based on mouse hepatocellular carcinomas in the NTP and NCI gavage studies is 1.4×10^0. This is derived as follows: the mean slope estimate from both studies, 1.1×10^{-2} $(mg/kg/day)^{-1}$, is multiplied by the molecular weight of 131.4 to give a potency index of 1.4×10^0. Rounding off to the nearest order of magnitude gives a value of 10^0, which is the scale presented on the horizontal axis of Figure 8-15. The index of 1.4×10^0 is among the least potent of the 54 suspect carcinogens, ranking in the lowest quartile. Ranking of the relative potency indices is subject to the uncertainties involved in comparing estimates of potency for a number of chemicals based on different routes of exposure in different species, using studies whose quality varies widely. All of the indices presented here are based on estimates of low-dose risk, using linear extrapolation from the observable range. These indices may not be appropriate for the comparison of potencies if linearity does not exist at the low-dose range, or if comparison is to be made at the high-dose range. If the latter is the case, then an index other than the one calculated above may be more appropriate.

8.6 SUMMARY

8.6.1 Qualitative Asssessment

8.6.1.1 Animal Studies--In a 90-week carcinogenicity bioassay with male and female B6C3F1 mice given estimated maximally (2,339 mg/kg in males and 1,739 mg/kg in females) and one-half maximally tolerated doses of technical grade, epoxide-stabilized TCI in corn oil daily, 5 days/week, for 78 weeks, statistically significant increases in the incidence of hepatocellular carcinomas in male and female mice compared to corn oil controls were found (NCI, 1976). Statistically significant increases in hepatocellular carcinoma incidence in treated male and female mice compared to vehicle-control mice were also observed in a repeat of the above carcinogenicity bioassay in which male and female B6C3F1 mice were treated with purified TCI, containing no detectable epoxides, in corn oil by gavage at a dose of 1,000 mg/kg/day, 5 days/week, for 103 weeks (NTP, 1982). In effect, this repeat study indicated that epoxide stabilizers were not necessary factors in the TCI-induced increases in hepatocellular carcinoma incidence in male and female B6C3F1 mice under the conditions of these bioassays. It is noted that spontaneous hepatocellular tumor formation occurs in the B6C3F1 mouse strain, particularly in the male.

A 110-week carcinogenicity study with male and female Osborne-Mendel rats given technical grade, epoxide-stabilized TCI in corn oil by gavage at estimated maximally (1,097 mg/kg) and one-half maximally tolerated doses daily, 5 days/week, for 78 weeks, was negative for carcinogenicity; however, this study may be inconclusive because of high mortality in the treatment groups (NCI, 1976). In a carcinogenicity study on male and female Fischer 344 rats treated with purified TCI, containing no detectable epoxides, in corn oil by gavage at estimated maximally (1,000 mg/kg) and one-half maximally tolerated doses for 103 weeks, a small incidence of renal adenocarcinomas in high-dose males at terminal sacrifice (3/16) was calculated to be a statistically significant increase over that in matched vehicle treated controls (0/33) by life table and incidental tumor tests, which adjust for mortality, but not by the Fisher Exact Test (NTP, 1982). However, the NTP, sponsor of the study, has concluded that this study is inadequate to evaluate the presence or absence of a carcinogenic response to TCI. Under the conditions of this study in Fischer 344 rats, there was a statistically significant increase in mortality in treated males and 2%, 6%, and 20% of the matched vehicle-control, low-dose, and high-dose males, respectively, were accidentally killed during the study. Toxic nephrosis characterized as cytomegaly was commonly found in the treated male and female Fischer 344 rats, with a stronger effect evident in males. A carcinogenicity study of purified TCI administered by gavage in olive oil to male and female Sprague-Dawley rats daily, 4 or 5 days/week for 52 weeks, at doses of 250 or 50 mg/kg followed by lifetime observation was negative, but the 52-week exposure period was below potential lifetime exposures for these animals (Maltoni, 1979).

Inhalation exposure of male Han:NMRI mice, male and female Han:Wist rats, and male and female Syrian hamsters to purified TCI vapor at levels of 100 ppm and 500 ppm 6 hours/day, 5 days/week, for 78 weeks followed by lifetime observation did not induce a carcinogenic effect (Henschler et al., 1980). Overt toxicity of TCI was not evident in rats and hamsters, which suggests that testing at higher levels of TCI was possible in this study, but mortality was greater in male mice compared to controls. A statistically significant increase in malignant lymphoma incidence was found in low-dose and high-dose female Han:NMRI mice compared to controls in this study; however, the spontaneous incidence of malignant lymphomas in controls was high (30%), and it was postulated in the study report that the increased incidence of malignant lymphomas in female mice could have been a result of an immunosuppressive response capable of enhancing susceptibility to tumor induction by specific, mostly inborn, viruses (Henschler et al., 1980).

A chronic inhalation study of technical grade, epoxide-stabilized TCI as a vapor was done in which male and female Charles River rats and male and female B6C3F1 mice were exposed to nominal levels of TCI at 100, 300, and 600 ppm 6 hours/day, 5 days/week, for 24 months (Bell et al., 1978). An audit of liver pathology revealed no carcinogenic effect in rats and a dose-related increase in the incidence of hepatocellular tumors in male and female B6C3F1 mice; however, this study is weakened by wide variability in the actual TCI exposure levels and lack of a concurrent matched control group of mice.

A series of studies done to evaluate the carcinogenic potential of purified TCI in lifetime treatment studies with female ICR/Ha Swiss mice included an initiation-promotion study in which a single topical application of 1 mg TCI in acetone was followed by lifetime repeated topical applications with the promoter phorbol myristate acetate; a study of complete carcinogenicity with repeated topical applications of 1 mg TCI in acetone; a study with once weekly subcutaneous injections of 0.5 mg TCI in trioctanoin; and a study in males as well as females with once-weekly gavage administrations of 0.5 mg TCI in trioctanoin (Van Duuren et al., 1979). Complete carcinogenic and initiating activities for TCI were not found in these studies. The dose used for topical application was below that estimated as maximally tolerated, but the authors concluded that no activity or low activity of chloroolefins tested for carcinogenicity on mouse skin would be expected because of a relatively low level of epoxidizing enzymes in mouse skin. More than once-weekly dosing by gavage and subcutaneous administration could have permitted a broader evaluation of TCI carcinogenicity.

Carcinogenic activity for TCI was not observed in several animal studies designed for an evaluation of general toxicity; however, these studies involved small groups and durations of treatment and observation that were less than the lifetime of the animals or, in one study, a duration that was not specified. Exposure of newborn Wistar rats for 10 weeks to 2,000 ppm TCI or vinyl chloride, 8 hours/day, 5 days/week, induced ATPase-deficient foci, reported as premalignant lesions, in liver only in rats exposed to the latter agent (Laib et al., 1978, 1979); however, while only vinyl chloride was effective with this exposure duration, it is not certain whether longer exposure to TCI could ultimately have induced similar foci.

The carcinogenic potential of TCI oxide, a putative direct-acting metabolite of TCI, has also been evaluated in lifetime treatment studies with female ICR/Ha Swiss mice (Van Duuren et al., 1983). These studies included an initiation-promotion study in which a single topical application of 1 mg TCI oxide in acetone was followed by lifetime repeated topical applications with phorbol myristate acetate; a study of complete carcinogenicity with repeated topical applications of 2.5 mg TCI oxide in acetone; and a study with once-weekly subcutaneous injections of 0.5 mg TCI oxide in trioctanoin. These studies were negative for initiating activity and complete carcinogenic activity at estimated maximum tolerated doses.

8.6.1.2 Cell Transformation Studies--Cell transformation activity by TCI, at minimally toxic doses, and 3-methylcholanthrene, a positive control agent, was found in an *in vitro* study with Fischer rat embryo cells (F1706, subculture 108) containing genetic information of the Rauscher leukemia virus (Price et al., 1978). Cell transformation has been developed as an end point for predicting carcinogenic activity; however, it is not certain whether these agents elicited transformation in this Fischer rat cell line through an action on the host cells, their integrated oncornavirus, or both.

Exposure of baby Syrian hamster kidney (BHK-211Cl 13) cells in the presence of rat liver microsomes *in vitro* to TCI solution in DMSO at doses including those producing toxicity did not induce cell transformation, whereas vinyl chloride induced cell transformation when administered as a gas at levels including those producing toxicity, but not as a solution in DMSO tested at levels where toxicity was not clearly evident (Styles, 1979). A broader evaluation could have been made with an additional test where cells were exposed to TCI as a vapor.

Cell transformation was induced by high concentrations (>1 mM) of TCI oxide capable of producing dose-related cytotoxicity in cultures of Syrian hamster embryo cells. The transformed colonies in this system were similar to those induced by benzo[a]pyrene and other chloroalkene epoxides (DiPaolo and Doniger, 1982).

8.6.1.3 Human Studies--Three cohort studies of workers exposed to TCI did not find that these workers experienced an excess risk of cancer (Axelson et al., 1978; Tola et al., 1980; Malek et al., 1979). Each of these studies, however, suffered from one or more of the following deficiencies: small sample size, lack of analysis by tumor site, problems with exposure definition, and problems with length of exposure.

A case-control study of malignant lymphoma cases provided some suggestion of an association of TCI exposure with malignant lymphoma (Hardell et al., 1981). However, failure by the authors to adjust for age and the possibility of inaccurate and biased exposure classification limit the usefulness of the results. A study of 56 liver cancer cases found no evidence of TCI exposure (Novotna et al., 1979); however, no controls were used in the study.

Paddle (1983) found that none of the primary liver cancer cases residing in an area near an industrial plant that produced TCI had been employed by the plant. This study had several deficiencies, and there are also limitations in the author's description of the study which make it difficult to evaluate. No controls were used in the study. The author provided no indication of the probability of finding a liver cancer case who had been an employee of the plant and had been exposed to TCI. Also, the author provided no information on the distribution of workers at the plant exposed to TCI by length and time of exposure. Furthermore, persons with liver cancer who may have worked at the plant but who did not live in the area of the plant or who had moved from the area were excluded from the study by study definition.

In summary, the epidemiologic data are inadequate for evaluating the carcinogenicity of TCI.

8.6.2 Quantitative Assessment

Four sets of gavage bioassay data on hepatocellular carcinomas in male and female mice (NTP, 1982 and NCI, 1976) are used to calculate the upper-bound slope estimate for TCI from the linearized multistage model. Since the upper-bound slope estimates calculated on the basis of these data sets are comparable, ranging from 5.8×10^{-3} to 1.9×10^{-2} mg/kg/day, the geometric mean, 1.1×10^{-2} mg/kg/day is used to calculate the incremental lifetime cancer risk (i.e., unit risk) due to a unit exposure of trichloroethylene in drinking water and in air. The upper-bound estimate of the cancer risk due to 1 μg/L of TCI in drinking water is 3.2×10^{-7}. The upper-bound estimate of the cancer risk due to 1 μg/m^3 of TCI in air is 1.3×10^{-6}. The relevant information on metabolism and kinetics for TCI by oral and inhalation exposures has been used to calculate the unit risks for drinking water and air.

None of the epidemiologic studies reviewed in this report provide positive evidence from which to estimate a unit risk for exposure to TCI. As an alternative, an upper-bound estimate has been calculated from one negative study. The calculation on the basis of human data results in a greater risk estimate than the estimate from animal data and thus does not appear to contradict the risk estimate calculated from the animal data.

The potency index of TCI is 1×10^0, ranking it in the lowest quartile of 54 chemicals that the CAG has evaluated as suspect carcinogens.

8.7 CONCLUSIONS

Evidence reviewed in this document for the carcinogenicity of TCI in experimental animals includes: increases in the incidences of hepatocellular carcinoma in male and female B6C3F1 mice (three studies), malignant lymphoma in female Han: NMRI mice, and renal adenocarcinoma in male Fischer 344 rats. The gavage treatment study of purified TCI in male and female B6C3F1 mice was done with basically the same experimental design as the gavage treatment study of technical grade TCI in male and female B6C3F1 mice to evaluate the role of epoxide stabilizers in the induction of hepatocellular carcinomas. Similar carcinogenic responses were observed in both studies. However, as discussed earlier in this chapter, a third study in B6C3F1 mice, the inhalation study, was weakened by deficiencies in its conduct. In the inhalation study in Han:NMRI mice, there was a 30% incidence of spontaneous lymphoma in control mice and the possibility of an indirect effect in treated mice; the gavage treatment study in Fischer 344 rats was considered by the study's sponsor, NTP, to be inadequate for a judgment of TCI carcinogenicity because of experimental deficiences (See Section 8.1).

While six epidemiologic studies or related surveys have focused on TCI exposure, the total body of evidence is considered to be inadequate to evaluate the carcinogenic potential. Three cohort studies and two surveys showed no excess risk of cancer resulting from exposure to TCI; however, in each case, study deficiencies limited their usefulness and/or diminished their sensitivity to detect a response. A case control study with a suggestive association between exposure and malignant lymphoma has flaws which limit the usefulness of the results.

Cell transformation activity by TCI in Fischer rat embryo cells containing rat oncornavirus was found, but it is not certain whether the effect could have occurred in the host genetic material, the oncornavirus, or both. As concluded in this document, currently available data provide suggestive evidence that commercial grade TCI is weakly active as an indirect mutagen, i.e., it requires metabolic activation. Data on pure TCI do not allow a conclusion to be drawn about its mutagenic potential; although if it is mutagenic, the available data suggest that pure TCI would be a very weak indirect mutagen. As discussed in the metabolism chapter herein, there is evidence that TCI metaolite(s) can react with cellular protein macromolecules *in vivo* and *in vitro* and with exogenous DNA and RNA *in vitro*. There are no known differences among species with regard to metabolic pathways or metabolic profiles for TCI. A study of purified radio-labeled TCI binding to DNA in liver *in vivo* in male B6C3F1 mice given a gavage dose of 1,200 mg/kg of TCI - ^{14}C(7,500 μCi/animal) suggested a low order of binding. Cell transformation with high doses of TCI oxide, a putative direct-acting carcinogenic metabolite of TCI, has been observed *in vitro*; however, animal studies of initiating and complete carcinogenic potential of TCI oxide were negative.

TCI is positive for the induction of malignant tumors of the liver in both male and female B6C3F1 mice in multiple studies. This constitutes a signal that TCI might be carcinogenic in humans. The positive lymphoma response in females of the Han:NMRI mouse strain does qualify as a separate strain, but that result is merely suggestive of a carcinogenic response because it occurs only in females, has a high spontaneous rate, and has been found to involve an indirect mechanism of action. The kidney tumor results in the NTP rat study are not strong indications of a response in a second species because of the small number of animals responding (3 of 49 animals) and because of the high mortality, although the statistical significance after mortality corrections suggests that a carcinogenic effect may be taking place.

The TCI carcinogenicity results could be classified under the criteria of the International Agency for Research on Cancer (IARC) as either "sufficient" or "limited" depending on which of the differing current scientific views about chlorinated organic compound induction of liver tumors in mice is chosen. Since there are no adequate epidemiologic data in humans, the overall ranking of TCI under the criteria of the IARC depends primarily upon the position taken regarding the mouse liver tumor. Thus, the overall ranking could be either Group 2B or Group 3. The more conservative public health view would regard TCI as a probable human carcinogen (Group 2B), but there is also scientific sentiment for regarding TCI as an agent that cannot be classified as to its carcinogenicity for humans (Group 3).

The U.S. Environmental Protection Agency's Proposed Guidelines for Carcinogen Assessment (U.S. EPA, 1984) regard the mouse-liver-tumor-only response as sufficient evidence for carcinogenicity in animals. A variety of factors such as lack of mutagenicity, low malignancy, and other such measures, if present, would downgrade this evidence; however, downgrading would not occur with the TCI results. Thus, based on EPA's proposed cancer guidelines, the overall evidence for TCI results in a classification of B2, i.e., a probable human carcinogen.

Additional carcinogenicity studies on purified TCI (no detectable epoxides) including those sponsored by the NTP with lifetime gavage treatment in four strains of rats (Osborne-Mendel, Marshall 540, August 28807, and ACI) and those performed in the laboratories of Dr. Caesar Maltoni with lifetime inhalation exposure in B6C3F1 mice, Swiss mice, and Sprague-Dawley rats are not finalized or published. The Fukuda et al. (1983) and Henschler et al. (1984) studies were not available at the time this document was submitted for public comment and Science Advisory Board review; thus, these studies have not been included in the final document but will be incorporated into any updates of this Health Assessment Document.

Gavage studies on hepatocellular carcinomas in mice provide a basis to calculate incremental lifetime cancer risks due to exposure to TCI. The development of the risk estimates is for the purpose of evaluating the "what-if" question: If TCI is carcinogenic in humans, what is the possible magnitude of the public health impact? The upper-bound estimate of the incremental cancer risk due to 1 μg/L of TCI in drinking water is 3.2×10^{-7}. The upper-bound estimate of the incremental cancer risk due to 1 $\mu g/m^3$ of TCI in air is 1.3×10^{-6}. The upper-bound nature of these estimates is such that the true risk is not likely to exceed this value and may be lower.

The potency index of TCI is 1×10^0, ranking it in the lowest quartile of 54 chemicals that the CAG has evaluated as suspect carcinogens.

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